資料2

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

１ 基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

２ 具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した**「資料概要A」**を事務局が作成し、送付する。

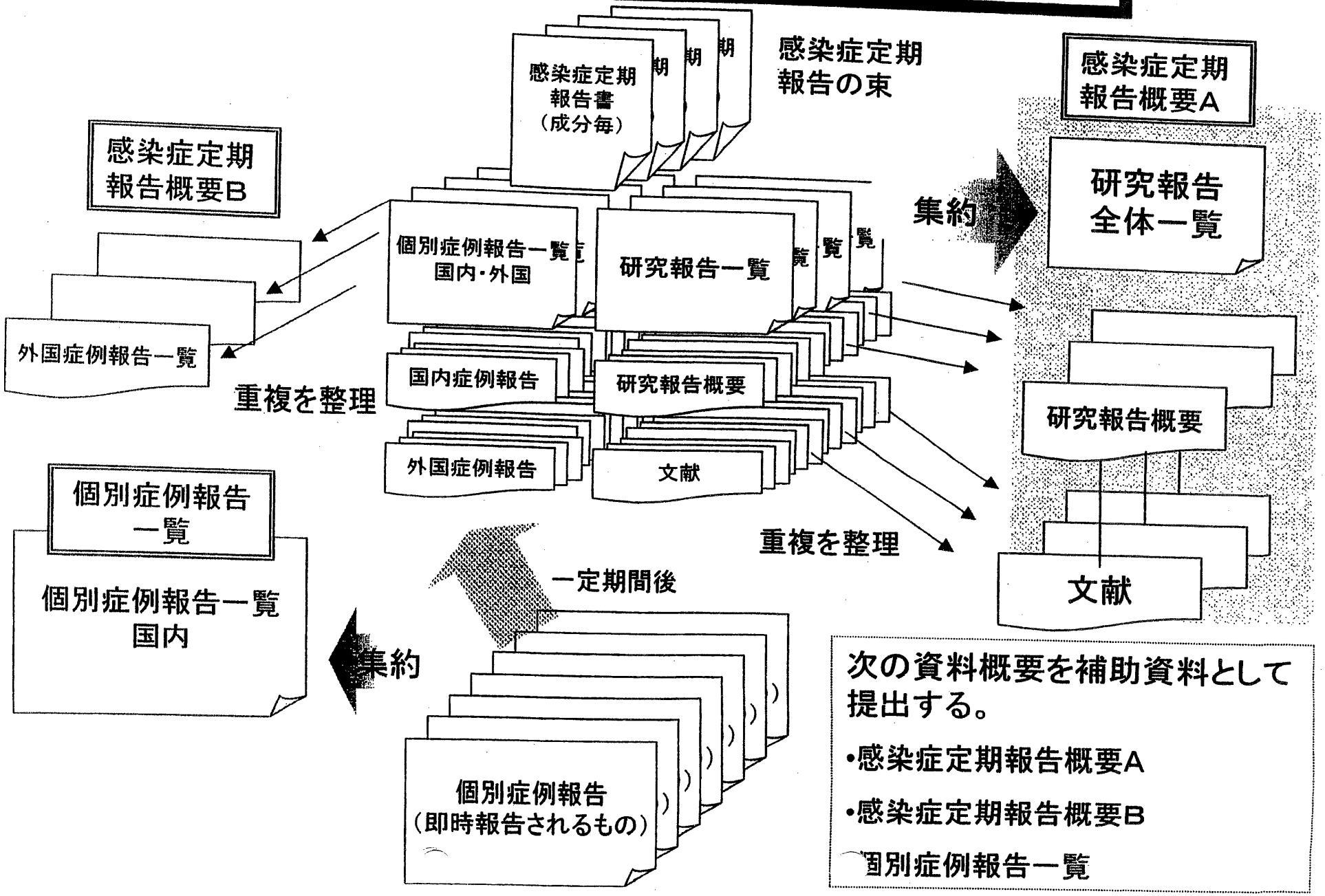
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症的報告について（目次）」資料は廃止することとする。

感染症定期報告・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

感染症定期報告概要

（平成２２年５月１８日）

平成２１年１２月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成２１年１２月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

２　一覧表においては、前回の運営委員会において報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

感染症定期報告の報告状況 (2009/12/1～2010/2/28)

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100070	2009/12/3	90774	A型肝炎	Eurosurveillance 2009 April 16; 14(15)	2008年9月1日-3月9日、スペイン・バルセロナにおいてA型肝炎に感染した150症例が報告された。この数は、前の2年の同時期と比べて3倍である。ほとんどの症例は、男性と性的関係を持つ男性(MSM)であることを報告した87名を含む、成人男性に発生した。これは、MSM集団におけるA型肝炎感染のアウトブレイクの可能性を示唆しており、感染リスクの高いコミュニティーへのより効果的なワクチン接種プログラムの必要性を強調している。	
100070	2009/12/3	90774	B・C型肝炎	Transfusion 2009; 49; 648-654	2005年8月、カナダ血液サービスは入れ墨や耳もしくは体のピアスに対する供血延期の期間を12ヶ月から6ヶ月に短縮した。本研究では、この変更が血液の安全性および安定供給に及ぼす影響を評価した。最近の供血者40,000名を対象とし、普及率を調べた結果、入れ墨、耳、体のピアスについてそれぞれ調査回答者の13.7、53.6、10.4%であり、過去6ヶ月以内の実施は最大0.7%であった。National Epidemiology Donor Databaseを用いて算出した供血延期期間変更前および後の感染症(TD)マーカー率は、100,000供血当たり21.6および19.2であった。症例対照試験はTD陽性供血者とマッチした対照者間のリスク因子を比較して行われ、最近の入れ墨やピアスはHCVまたはHBVのリスク因子ではなかった。延期期間の短縮により、供血延期の件数は入れ墨で20%、ピアスで32%減少した。供血期間の短縮後、検出できるほどの安全性に対する影響は少なく、血液供給においては期待効果以下ではあるが有効であった。	
100070	2009/12/3	90774	B型肝炎	Hepatology 2009; 49; S156-165	B型肝炎の再燃とは、非活動型もしくはB型肝炎が治癒した患者にB型肝炎ウイルス(HBV)の急激な増幅が起きることである。最も説明が成されている例として、B型肝炎の再燃はリンパ腫または白血病の癌化学療法を受けている非活動性もしくはほとんど活動していないB型肝炎表面抗原(HBsAg)キャリアに起きている。通常は化学療法の間血清中HBV DNAが上昇し、化学療法中止後に免疫再構築による疾病増悪およびHBV DNAクリアランスと続く。いくつかの無作為化プラセボ対照試験は、抗ウイルス剤の予防投与によって再燃を防ぐことができることを示した。癌化学療法や移植を行うHBsAg陽性者にルーチンの予防が推奨されるが、HBsAgスクリーニングを行う患者の選定や使用する抗ウイルス剤の種類や期間、およびHBsAg陰性のB型肝炎治癒患者への予防など疑問はある。再燃の分子生物学的メカニズムや異なる患者集団における診断、治療および予防の最適化についての研究が望まれる。	
100070	2009/12/3	90774	B型肝炎	Transfusion 2009 July; 49; 1314-1320	HBsAg(hepatitis B surface antigen)に陽性を示した供血者とHBV(hepatitis B virus)感染者とのHBVgenotypeを比較するため、HBsAg陽性供血者の遺伝子型を決定した。2006年10月-2007年9月の日本人供血者のデータは日本赤十字社から提供を受け、1887例についてHBVの主な6genotypes(A-F)をELISA(enzyme-linked immunosorbent assay)法によって決定した。HBsAg陽性ドナーについてHBVコア抗原に対するIgM抗体の有無の確認を行った。供血者と患者間で示されたHBVgenotype分布における有意差はC/B遺伝子型比で認められ、この比率は供血者で低く(2.0-3.9)、患者で高かった(5.3-18.2)。また、genotypeBの比率は10歳代の13.8%から増加し、50歳代では42.4%であったが、genotypeC比率は10歳代の83.1%から50歳代の55.1%に減少した。HBcAgに対するIgM抗体およびNAT(nucleic acid test)両者に陽性であるドナーでは、genotypeAおよびBは男性のみであった。日本人供血者におけるHBVgenotypeの年齢特異的な分布は、B/C遺伝子型比に特徴があり、米国もしくは西欧諸国由来であるHBVgenotypeAの性特異的分布は、日本人男性ドナーに観察された。	

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100075	2009/12/18	90810	B型肝炎	日本肝臓学会大会第13回 2009; A536 (2009 October 14-15) (日本消火器病学会抄録 106 A536 肝S3-13)	輸血後検査におけるHBV陽性例の発生状況とその原因について全国調査を行った。2007年1-12月の輸血後検査におけるHBVDNAまたはHBs抗原陽性例経験の有無を問い、有経験施設には個別調査を行った結果、輸血後ＨＢＶ陽性例の経験施設(37)のうち18施設が37症例を回答した。輸血前（保管）検体の検査結果と献血者保管検体の個別NAT検査の成績を元に、既感染例、輸血感染例、再活性化例、その他、分類不能の5分類に該当する症例は、それぞれ19、4、6、0、8例であり、輸血を要する治療を行った患者にHBV活性化が存在することが判明した。輸血によるHBV伝播とHBV再活性化の鑑別には、輸血前のHBs･HBc抗体検査か輸血前検体保管が必要である。	1
100070	2009/12/3	90774	E型肝炎	Emerging Infectious Disease 2009; 15 ;704-708	E型肝炎ウイルス（HEV)のgenotype3は日本においては不顕性感染とされているが、重篤な肝炎を発症した国内8症例について、強毒性をもたらすHEVの遺伝的特徴を解析するため遺伝子配列を決定した。系統樹解析の結果、いずれも他のgenotype3とは区別され、JIO株と名付けられた固有のクラスターに分類された。このJIO関連ウイルスは他のHEVgenotype3とは異なる18のアミノ酸をコードしており、また、JIOクラスターのヒトHEV株のほぼすべてに共通する置換はヘリカーゼ領域(V239A)に位置し、V239Aはgenotype4では一般的であることから、毒性の増強と関連が示唆された。また、genotype3に属するswJ19株に感染した5匹のブタから遺伝子を解析した結果、同様にヘリカーゼにV239A置換が存在していたことから、JIO関連ウイルスが人獣共通であることが疑われた。	
100070	2009/12/3	90774	E型肝炎	第57回日本輸血･細胞治療学会2009; 55; 244 (日本輸血細胞治療学会誌 55(2) O-051)	北海道で献血者のHEV感染の実態を解析するため、2005年1月-2008年11月に北海道内の献血者1,075,793名について20本プールによるHEV NATを実施した。HEV　NAT陽性者は140名であり、献血時のHEV抗体保有率は3割以下、感染初期の献血が多かった。陽性者のHEVのgenotypeは9割以上が3型で4型も認められた。陽性者の約7割は献血前に動物内臓肉の喫食歴があり、陽性者の半数にはその後ALT値の上昇が見られた。北海道内の献血者集団に於けるHEV　RNA陽性頻度は高く、zoonotic infectionが起きていると考えられる。	
100070	2009/12/3	90774	HHV-8感染	Journal of Infectious Disease 2009; 199(11); 1592-1598	米国内で輸血を介したHHV-8感染の調査を行った。供血者-受血者のペアを明確にした米国内調査を行うため、1970年代に登録されたTTVS（Transfusion-transmitted Viruses Study）の参加者にHHV-8血清学的検査を行った。HHV-8抗体陽性率は、供血者では2.8%、受血者では7.1%、輸血されず手術を行った対照患者では7.7%、カポジ肉腫のある対照患者では96.3%であった。1例の受血者はセロコンバージョンしたが、この患者にはHHV-8陽性の血液ユニットは輸血されなかった。また、輸血されず手術を行った対照患者1例もセロコンバージョンした。セロコンバージョン率は、受血者が1.6（1000人-年あたり）であり、輸血を受けていない手術を行った対照患者では3.6（1000人-年あたり）であった。輸血群と非輸血群におけるHHV-8セロコンバージョン率には統計学的な差はなく、かつ過去の集団の特徴（例：白血球除去施行前）は現在の輸血を介した伝播が稀であることを示している。	

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100070	2009/12/3	90774	HIV	FDA/CBER 2009 August Guidance for Industry	2009年8月米国FDAは、ヒト免疫不全ウイルス1型(HIV-1)グループOの感染リスクの高いドナーの管理に関する勧告と題した企業向けガイダンスを発表し、即時適用するよう求めた。 A.HIV-1グループO感染リスクの高い供血者を特定するために問診事項が改定された。以下の質問を供血者問診票(donor history questionnaire)のハイリスク行為についての質問に盛り込むこと。 1.1977年以降、以下の国で生まれたかもしくは居住していたことがあるか：カルメーン、ベナン、中央アフリカ共和国、チャド、コンゴ、赤道ギニア、ケニヤ、カボン、ニジェール、ナイジェリア、セネガル、トーゴ、ザンビア。それはいつか。 2.1977年以降にこれらの国へ渡航歴がある場合、輸血や血液製剤による治療を受けたか。それはいつか。 3.1977年以降にこれらの国で生まれたヒトもしくは居住していたヒトと性的接触を持ったか。それはいつか。 質問のいずれかを肯定した感染の可能性のある供血者を無制限に供血延期とすること。ただし、最後のHIV-1グループOの曝露から1年後に、以下Cの勧告に従って再エントリーを検討できる。 B.HIV-1グループO抗体の検出感度を有するとラベルのIntenden Use項に記載された、供血者スクリーニング用の承認済み抗HIV-1/2テストを実施する場合、上記Aの問診を中止してもよい。 C.HIV-1グループO感染リスクの質問への回答に基づき供給延期とされた供給者の再エントリーについて、最後のHIV-1グループOへの曝露から最低でも1年の保留期間を経た後、供給者は以下の場合、再エントリーしてよい。 1.当該供血者の現在の供血時に、HIV-1グループO抗体の検出感度を有するとラベルのIntenden Use項に記載された抗HIV-1/2スクリーニングテストの結果、陰性と判明し、かつ 2.当該供血者が全ての供血者適格基準を満たしている。	2
100081	2010/1/8	90851	HIV	Nature Medicine 2009; 15(8); 871-872	2001年以降、フランスのレファレンス研究所はHIVの遺伝子多様性を調査しており、2004年に血清検査でHIV陽性であった62歳の女性の血清試料 (RBF168)を分析した。この血清は女性がカメルーンからパリに移住した直後に採取された。女性は現在AIDSの症状はない。RBF168からウイルスを分離し、ウイルス遺伝子を解析した結果、RBF168はゴリラのサル免疫不全ウイルス (SIVgor) と最も近縁であった。この新しいウイルスは新しいHIV-1のプロトタイプであると思われるが、HIV-1のグループM,N,Oとは異なり、グループPと命名された。RBF168株が発見される前は、HIVグループOが最もSIVgorに近縁であったが、変異の大きさから現在のSIVgorから直接出現したのではなく、SIVgorのゴリラからヒトへの伝播が起因していると考えられた。これらの結果より、HIVの感染源としてチンパンジーに加えてゴリラが示された。	
100070	2009/12/3	90774	HIV	Pediatrics 2009; 124; 658-666	米国において9、15および39ヶ月の子供3例は、臨床症状から検査が行われた結果、HIV感染と診断された。2例については、母親がHIV感染者であるが、母乳は与えず、また周産期感染は否定された。3例目は、母親ではなく養育していた叔母がHIV感染者であった。全例とも、HIV感染者である養育者が食べ物を噛んで与えており、2例では噛み与えた大人に口腔内出血があった。EnvのC2V3C3またはgp41コード領域とgagのp17コード領域を用いた系統発生解析の結果は、3例中2例は養育者の噛み与えによってHIV感染が起きたという疫学的結論を支持した。	
100070	2009/12/3	90774	HIV	日本感染症学会第83回総会 2009 April 23-24; 314 (日本感染症学会抄録 p.314 P-102)	名古屋医療センターにおいて、4例にHIV-2の感染が疑われた。HIV抗体陽性かつ血中HIV-1RNAコピー数が検出限度以下を示した4例 (外国籍男性3例、日本国籍女性1例) の末梢血白血球より抽出したDNAを鋳型にPCRによりgagおよびenv領域の増幅後、遺伝子配列を決定した。4例中3例はHIV-2であることを確認し、日本国籍女性については確定診断に至らなかった。解析に成功した3例の内、1例はサブタイプA,他の2例はサブタイプ判定には至らなかった。日本国内においてもHIV-2 のスクリーニングを強化する必要がある。	

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100070	2009/12/3	90774	ＨＴＬＶ	47 news. 2009 Jun 27	厚生労働省研究班は2006-2007年に初めて献血した全国約119万人を対象に、HTLV-1の調査を実施した結果、3787人の感染が確認され、国内感染者数は約108万人と推計した。約20年前の前回調査の120万人と比べて大きな変化はなかった。研究班班長である山口一成国立感染症研究所客員研究員は、感染者の地域別割合の高かった九州で減少し、大都市圏（関東・中部・近畿）で増加したが、これは感染者が多い九州からの人の移動が背景にあると指摘した。	
100070	2009/12/3	90774	Q熱	Eurosuveilance 2009; 14(19); 2009 May 14	オランダでは2007および2008年のアウトブレイク後再びQ熱報告が2009年4月から急増し、1月1日-5月11日の間、総計345症例が報告された。男女比は約1.7:1で、年齢中央値は49（38-61）歳であった。ほとんどの患者が2007および2008年の報告と同様、Noord-Brabant地方の同地域の住民であるが、感染領域は拡大傾向にある。オランダにおけるQ熱の主な臨床症状は肺炎であり、2008年に報告された患者は、545例が肺炎、33例が肝炎、115例が他の発熱性疾患を発症した。Noord-Brabant地方には大規模なヤギ農場が集中しており、流産の増加している農場が発生源と疑われる。小型反芻動物へのワクチン接種義務化が始まっており、2010年には効果が出ると考えられる。	
100070	2009/12/3	90774	アメリカ・トリパノソーマ症	CBER（http://www.fda.gov/cber/gdlns/chagas.htm)	CBERから、輸血用全血、血液成分製剤、ヒト細胞・組織及びヒト細胞・組織由来製剤のTrypanosoma cruziが伝播する危険性を低減するための血清学的検査実施についてのガイダンス案を公表。	
100070	2009/12/3	90774	アメリカ・トリパノソーマ症	Emerging Infectious Disease 2009; 15:653-655	ブラジルで2006年1～11月に発生したアメリカ・トリパノソーマ症のアウトブレイク（178症例）について、調査の結果、アサイー果実を潰す際に、原虫を媒介するサシガメの排泄物が混入した可能性が考えられた。	
100080	2009/12/22	90825	アメリカ・トリパノソーマ症	FDA Guidance for Industry(draft) "Use of Serological Tests to Reduce the Risk of Transmission of Trypanosoma cruzi Infection in ・・・・	Trypanosoma cruzi抗体検出用のELISA検査システムがCBERにより許可されたことをうけ、米国において、全血、血液成分及びHCT/Psにおけるトリパノソーマ症伝播のリスク低減のためのドナースクリーニングについて、FDAよりドラフトガイダンスが公表された。最終版発表後1年以内にこのガイダンスに適合することが推奨されることとなる。	
100070	2009/12/3	90774	アメリカ・トリパノソーマ症	ProMED-mail 20090406.1328	ベネズエラでグアヴァジュースの摂取によるアメリカ・トリパノソーマ症のアウトブレイクが発生し、同学校に通う児童47名と教師3名が感染。児童3名が死亡。	
100075	2009/12/18	90810	アメリカ・トリパノソーマ症	日本感染症学会第58回東日本地方会 2009; 124 041 (2009 October 30-31) (日本感染症学会抄録 p.124 041)	近年、各地医療機関から依頼のあった心疾患患者41名についてシャーガス病原体Trypanosoma cruzi(T.cruzi)血清抗体検査を行った結果、15名が明らかに陽性を示し、シャーガス病が示唆された。更に抗体陽性者血液からT.cruzi-DNAを検出し、また、血液培養の結果2名からT.cruzi虫体を分離した。慢性の病原体キャリアーが日本に存在することが明らかとなったが、媒介昆虫の存在しない国内において、感染経路は二次感染であるため、事前の抗体検査で防ぐことが出来る。	3

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100075	2009/12/18	90810	ウイルス性脳炎	Emerging Infectious Disease 2009; 15; 1671-1672 (October 2009)	2008年7月、オーストリア東部の山岳地帯で6例が感染したTBE (Tick-born encephalitis) アウトブレイクの調査が行われた。初発患者の羊飼いは、高山牧場地に24日間滞在後、髄膜炎の臨床症状を呈し、TBEV (TBE virus)感染陽性と確定された。患者はダニに咬合された記憶はなく、発症8-11日前に非殺菌のヤギ乳および牛乳から製造された自家製チーズを食べていた。同じチーズを食べた6名中5名がTBE感染と診断され、非感染であった1例はチーズを食べた直後嘔吐していた。チーズはヤギ1頭およびウシ3頭の乳から製造されたが、そのヤギはHIおよび中和抗体検査でTBEV陽性であり、ウシ3頭は抗体陰性であった。また、ホエイおよびヤギ乳を与えられ、同じ牧草地で飼育されていたブタ4頭がTBEV抗体陽性を示した。このアウトブレイクは、中央ヨーロッパ高地におけるTBEの新興と、TBE経口感染の高い効率性を示した。	4
100075	2009/12/18	90810	ウイルス感染	Journal of General of Violigy 2009; 90; 2644-2649	1996年、インドケララ州で発生した脳炎アウトブレイクの調査において、蚊 (Culex tritaeniorhynchus) のプールからアルボウイルスが分離された。補体結合検査より日本脳炎とウエストナイルウイルスに交差反応を示すアルボウイルスの特徴が示され、アルボウイルス分離株に対する過免疫血清を使用したプラーク減少 中和反応検査の結果、血清は日本脳炎ウイルスでは陽性を示さず、ウエストナイルウイルスで弱陽性であった。このアルボウイルスはバガサウイルス(BAGV)の特徴を示し、脳炎患者の血清は15%(8/53)がBAGV中和抗体陽性を示した。インドからの初のBAGV分離の報告であり、また、人間集団がBAGVに曝露されていることが示唆された。	5
100070	2009/12/3	90774	ウイルス感染	PLoS Pathogens 2009; 4: e1000455	2008年に南アで発生した致死性出血熱のアウトブレイクにおいて、30年ぶりに新規の旧世界アレナウイルスが分離された。発見された地名 (Lusaka, Johannesburg) より、Lujo virusと命名された。	
100070	2009/12/3	90774	ウイルス感染	ProMED-mail 20090806.2782	2009年8月4日、ブラジルMazagaoで過去3ヵ月間に657例がオロポーチ熱に感染した事を当局は発表した。このうち29例はIEC(Instituto Evandro Chagas)によって確定診断がなされ、この病気の原因はCulicoides属ヌカカによる刺咬であると分かった。症状はデング熱やマラリアに似ており、発熱、頭痛およ全身性筋肉痛である。初発例は2009年3月に発生し、4月および5月には報告が激増し、MazagaoのVelhoおよびCarvaoで600を超えた。オロポーチウイルスはブラジルで2番目のアルボウイルス熱の原因ウイルスであり、ブラジルでは過去30年間に約50万人の発熱例が起きている。オロポーチ熱のアウトブレイクはアマゾン地域でのみ報告がある。	
100070	2009/12/3	90774	ウイルス感染	日本感染症学会第83回総会 (日本感染症学会抄録 P224 O-171)	2007年に初めて報告された新興感染症コウモリオルソレオウイルス (別名：マラッカウイルス) による急性上気道炎の報告である。2007年11月にインドネシア・バリ島から帰国した男性は帰国数日前から発熱、関節痛が出現し、帰国後も強い上気道炎症を呈し、オルソレオウイルス感染症と判明した。本ウイルスはコウモリを宿主とし、本患者はコウモリとの接触はなかったが、渡航先で上気道症状を呈する現地住民との接触があった。本患者では回復期に抗体が検出されたが、他の接触者は全て陰性であった。	
100070	2009/12/3	90774	ウエストナイルウイルス	CDC (http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount08_detailed.htm)	2008年、米国におけるウエストナイルウイルス感染症例は46州から1356例が報告され、うち687例では脳炎や髄膜炎を発症、死亡に至ったのは44例だった。	

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100075	2009/12/18	90810	ウエストナイルウイルス	Emerging Infectious Disease 2009; 15; 1668-1670 (October 2009)	WNV(West Nile virus)感染状況と2003-2008年に供給された米国製血漿由来静注用免疫グロブリン製剤(IGIV)における中和抗体価の関係が調査された。WNVは1999年に米国に持ち込まれたが、2003年にIGIVのWNV中和抗体平均値が顕著に上昇し、米国人口の0.5%がWNVに感染したと推定された。また、米国の人口における既感染者の割合は、毎年0.1%増加し、IGIVの中和抗体価平均値と概ね相関があった。2008年に出荷されたIGIVの中央抗体価は平均21(n=256)であり、NATでWNV感染を確定したヒトから得られた血漿では更に高い抗体価 (平均208(n=30))であった。血漿中IgG濃度を補正し、IGIV調整濃度10%と比較すると血漿試料はIGIVより100倍高値であった。この結果は、WNV既感染者は米国人口の1%であると推定したこれまでの報告と一致した。	6
100084	2010/1/26	90859	ウエストナイルウイルス	FDA/CBER Guidance for Industry 2009 November	2009年11月、FDAは企業向けガイダンス、「輸血目的の全血および血液成分の供血者からのWNV(West Nile Virus)感染リスクを減じるためのNAT(Nucleic Acid Tests)の使用」を発表した。勧告(Recommendation)の内容は、 A. 検査、ユニット管理および供血者管理：1. 輸血目的の全血および血液成分の供血サンプルにつき、承認されたNAT (MP-NATもしくはID-NAT)を用いてWNVの通年検査を行うこと。WNVの高活動地域ではID-NAT (individual donation)を推奨する。 2. MP-NATによる検査の結果、陰性であったミニプールを構成していた検査サンプルのユニットは出荷できる。ミニプールがNAT陽性を示した場合には、ID-NATを用いて各サンプルを検査し、陽性を示したユニットを特定すること。 a. すべてのID-NATで陰性であったユニットは出荷できる。 b. 個別献血が陽性であった場合、そのユニットは廃棄し、120日間の供血延期とし、 該当献血から120日前の期間における製品の回収および貯留を推奨する。 3. ID-NATを用いた検査を実施する場合には、A1．2aおよび2bの手順に従う事を推奨する。 B. MP-NATからID-NATへの切り替え：1. 血液を収集する地域でのWNV活動が高いことを定義する基準を確立し、バリデートすること。2. 血液を集める地域でのWNV活動が高い間、MP-NATからID-NATへ切り替える閾値を設定し、また、活動が収まった際にMP-NATに戻す閾値を設定すること。3. 実行可能になり次第、ただし、閾値到達から48時間以内に、MP-NATからID-NATに切り替える。4.この決定に関するSOPを作成し、従うこと。 C. 検査実施の報告 D. 輸血目的の全血および血液成分の表示	7
100095	2010/2/9	90920	感染	Google News 2009 Decmber 18	2009年12月18日、臓器提供者から少なくとも1人の臓器移植者に極めて珍しい感染が認められ、初のアメーバ(Balamuthia mandrillaris)のヒト-ヒト感染が報じられた。11月にUMMC (University of Mississippi Medical Center)で神経障害で亡くなった患者から臓器提供を受けた4例のうち、2例は重症（それ以外は無症状）であり、CDCは1例にBalamuthia mandrillarisを確認した。この微少寄生虫は土壌で発見され、ヒト、ウマ、イヌ、ヒツジおよび霊長類に脳炎を引き起こす。免疫抑制状態にある臓器移植患者では危険な寄生虫である。ヒト感染は極めて珍しく、1990年の発見後、世界で150例のみが報告されている。	8
100070	2009/12/3	90774	感染症	第57回日本輸血・細胞治療学会 2009; 55; 245 (日本輸血細胞治療学会誌 55(2) O-053)	日本赤十字社が2008年に収集し、報告した輸血関連感染（疑）症例149例の現状と解析結果である。149例の病原体別内訳は、HBV61例、HCV38例、細菌46例、HEV2例、HIV1例およびCMV1例であった。HBV4例、HEV2例および細菌2例については献血者検体から病原体を検出し、いずれも輸血と感染症との因果関係は高いと評価された。また、輸血後B型肝炎を発症した1例は、劇症肝炎により死亡した。日赤では2008年8月よりCLEIA法および新NATシステムを導入し、安全性の向上に努めている。	

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100070	2009/12/3	90774	細菌感染	日本感染症学会第83回総会 2009 April 23-24; 224 O-172 (日本感染症学会抄録 p.224 O-172)	2002-2003年に高知県で日本紅斑熱が疑われた患者18名の保存血液を解析した結果、2名からヒトアナプラズマ(Anaplasma phagocytophilum:A.p.)に特異的なp44/msp2遺伝子が検出され、ヒトアナプラズマ症の国内における存在を初めて確認した。1例はヒトアナプラズマ症で、もう1例はA.p.と日本紅斑熱リケッチア(Rickettsia japomicar.R.j)の混合感染症であった。	
100070	2009/12/3	90774	チクングニヤウイルス感染	CDC 2009 August 17	2009年8月17日、米国CDCはアジアでのチクングニヤ熱のアウトブレイクに注意喚起をした。2009年1月以降、チクングニヤ熱症例数の増加がアジアの一部で報告されている。チクングニヤ熱は感染した蚊を介してもたらされるウイルスによって発症し、突発性発熱、関節痛、悪寒、頭痛、吐き気や発疹などを伴う。タイでは2009年7月22日現在、南部でアウトブレイクが起こり、34，200超の症例（死亡例なし）が報告され、マレーシアでは2009年7月18日現在、2900症例、インドでは2009年4月29日現在、2700例の疑い症例（死亡例なし）が報告された。渡航者へのアドバイスとして、チクングニヤ熱を防ぐ薬物治療やワクチンはないため、CDCは、虫除けを使用し、蚊にさされないよう自己防衛し、発症を自覚した際には、医療機関を受診するよう奨めている。	
100075	2009/12/18	90810	チクングニヤウイルス感染	日本感染症学会第58回東日本地方会 2009; 124 041 (2009 October 30-31) (日本感染症学会抄録 p.124 041)	2009年5-6月、東南アジアから帰国後関節痛を主訴に来院した3例はチクングニヤウイルスIgM抗体および中和抗体陽性であり、血清学的にCHIKF(Chikungunya fever)と診断された。3例はそれぞれインドネシア・スマトラ島、インドネシア・ジャワ島もしくはマレーシア・クアラルンプール郊外に渡航し、いずれも現地で発熱および関節痛が出現した。解熱したが帰国後も関節痛は持続し、受診に至った。	9
100070	2009/12/3	90774	デング熱	ProMED-mail 20090831.3065	ベトナムハノイ市では、デング熱症例が深刻な増加を示しており、2009年初から8月下旬までに2500症例が報告され、これは2008年の同時期と比べて10倍以上であった。また、ホーチンミン市ではデング熱症例数の急増はないものの、多くの患者が重症化しており、死亡例も多くなっている。同市の第一小児病院は、毎日20-25人がデング熱症例のため来院しており、小児のデング熱症例は、感染後1-2日は手足口病やH1N1インフルエンザとの判別が難しいためデング熱への警戒をゆるめることがあるが、小児は死に至ることがあると注意喚起した。	10
100079	2009/12/18	90814	パルボウイルス	FDA/CBER Guidance for Industry 2009 July	FDAが血漿由来製品の製造業者向けに提供するガイダンス。血漿由来製品の製造に使用される原料血漿および転用血漿用の製造過程において、ヒトパルボウイルスB19を検出するための核酸増幅検査（nucleic acid test;NAT)を行う事を推奨している。すべての血漿由来製品について、製造プール中のパルボウイルスB19DNAのウイルス負荷が10000IU/mLを超えない事を保障するために、 ○すべての血漿由来製剤に対し、製造用プール血漿中のHPV B19 DNAの濃度が10000IU/mLを超えないように、工程内検査としてHPV B19 NATを実施すべきである。 ○血漿由来製剤の製造に投入する血漿ユニットのスクリーニングには、ミニプールサンプルに対してHPV B19 NATを実施すること。HPV B19 NATで用いるプライマーおよびプローブは、このウイルスの既知のすべての遺伝子型を検出できるものを用いること。 ○ 血漿由来製剤の製造に投入する血漿ユニットに、製造用プール血漿のHPV B19 DNA濃度が10^4IU/mLを超えるような高値を示すものが見つかった場合は、当該血漿ユニットは使用しないこと。	
100080	2009/12/22	90825	パルボウイルス	Transfusion (Malden) 2009; 49(7): 1488-1492	米国において、パルボウイルスB19（B19V)のGenotype3がアメリカ人供血者から初めて検出された。B19Vを検出するための広範囲な特異性のあるPCRを用い、81,000人以上の供血者から集めた約440,000の臨床サンプルを調べ、更にはB19Vタイター とDNA解析および抗体濃度を調べた。この評価の結果、DNA配列分析によってB19VGenotype3に感染していると確認された米国人1人のドナーから28日の間に8回の血漿ドネーションを行っていることが明らかとなった。ウイルス価はピーク時で10^11IU/MLを示し、下がるに連れてIgMレベルが上昇し、IgGレベルは約7日遅れて上昇した。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
100070	2009/12/3	90774	ボリビア出血熱	Emerging Infectious Disease 2009; 15; 1526-1528 (September 2009)	BHF(Bolivian hemorrhagic fever)は1959年にボリビア東部でのアウトブレイク発生時に初めて報告され、2007年2-3月、ボリビアで少なくとも20例（死亡3例）のBHF疑い例が報告された。2008年2月には少なくとも200例（死亡12例）の疑い例が報告され、19症例の血清を間接免疫蛍光法およびPCRを用いて検査した。その結果、アレナウイルス5株が分離され、ウイルスRNAの遺伝子配列の結果、マチュポウイルスを確認し、8つの主要な系統に分類された。その後も、マチュポウイルスは孤発症例やボリビアでのBHFアウトブレイクの原因となっているが、5例（死亡3例）の農業従事者である患者については、5例ともBHF感染歴のある患者からの血漿成分輸血を受けたが、3例は死亡した。病状が重篤化する前に、マチュポウイルスによって免疫が惹起された血漿を投与することが生存率を高くするかもしれない。	11
100080	2009/12/22	90825	マラリア	Clinical Infection Deiseases 2009; 49; 852-860	ヒトにおけるPlasmodium knowlesi感染の臨床的な特徴および検査結果を調べる目的で、急性P. knowlesi感染患者の背景と経過について系統的に調べ、2006年7月-2008年2月に、Kapit病院でPCRにより急性マラリアと確定された、治療歴の無い非妊娠成人から臨床データおよび検査結果を収集した。152例のうち、P.knowlesi、P.falciparumもしくはP.vivaxに感染した症例は107(70%)、24(16%)および21(14%)であり、非特異的発熱症状のあるP.knowlesi感染患者の入院時寄生虫値の中央値は1387parasite/ulであり、全例が血小板減少を示した。ほとんどのP.knowlesi感染患者には合併症はなく、クロロキンおよびプリマキン治療で治癒した。WHOの熱帯性マラリアの判断基準により7人は重症であった。入院時のP.knowlesi寄生虫血症は呼吸困難の独立した決定因子であり、入院時の血清クレアチニンレベル、血清ビリルビンおよび血小板数と同様であった。2例のP.knowlesi感染患者が死亡し、死亡率は1.8%(95%信頼区間、0.2-6.6%)であった。P.knowlesiは広範囲の疾病を引き起こすが、多くの場合合併症伴わず、治療に速やかに反応し、約10人に1人が死亡を伴う合併症となる。	
100080	2009/12/22	90825	リケッチア症	日本感染症学会第83回総会 2009 April 23-24 (日本感染症学会抄録 p.214 O-152)	平成20年8月、仙台市においてリケッチア症を疑う患者が発生した。生検材料を用いたPCRにより陽性であったが、シークエンス解析により、ロシアや中国の患者から報告されているR.heilomgiangensisに一致した。国内に、日本紅斑熱とは異なる紅斑熱ケッチア症が存在することが示された。	
100075	2009/12/18	90810	レトロウイルス(XMRV)	Sience 2009; 326; 585-588	慢性疲労症候群（CFS：Chronic Fatigue Syndrome)患者の血液細胞に感染性レトロウイルスXMRV(xenotropic murine leukemia virus-related virus)を検出した。CFSは原因不明の衰弱していく疾患で、世界中で1700万人が罹患していると推定されている。CFS患者の末梢血単核球を調べた結果、ヒトgammaretorovirusであるXMRVのDNAが、患者101例中68例（67%）に検出され、健康対照者では218例中8例（3.7%）であった。細胞培養の結果、患者由来のXMRVは感染性があり、ウイルスの細胞を介したおよび無細胞性感染のいずれも可能性が示された。CFS患者由来の活性化PBMC、B細胞、T細胞に曝露した後、非感染初代培養リンパ球および指標細胞培養系には二次感染が認められた。これらの結果は、XMRVがCFSの病原性における要因となる可能性を示唆した。	12
100070	2009/12/3	90774	インフルエンザ	Virus Res. 2009; 140; 85-90	中国のブタからヒト様H1N1インフルエンザウイルスが検出され、ブタがヒトにおけるパンデミックを引き起こす古典的なインフルエンザウイルス保有宿主である証拠が示された。	

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100080	2009/12/22	90825	インフルエンザ	日本ウイルス学会第57回学術集会 1P074 (p.355) (日本ウイルス学会抄録 p.355 1P074)	日本で採血された血漿を原料として製造された静注用グロブリン製剤(IVIG)にClassical Swine Influenza A(H1N1) virusおよびInfluenza A(H1N1) pdm virusに反応する抗体が含まれているか調べ、ドナーが免疫を獲得している可能性について検討した。その結果、IVIGにブタおよび新型ウイルスに対するHIおよびNT活性がそれぞれ8倍、64倍と認められ、日本において、ある程度の率でInfluenza A(H1N1) pdm virusに反応する抗体を保有するドナーが存在すると推測された。	13
100080	2009/12/22	90825	新型インフルエンザ(H1N1)	CDC/MMRW 2009; 58: 521-524	05～06年、06～07年、07～08年の季節性インフルエンザワクチン接種コホートの保存ペア血清を用いて、新型インフルエンザウイルスの交差反応性を検討した。18‐64歳ではワクチン接種前に6～9%、60歳以上では33%が交差反応を示した。ワクチン接種後には交差反応を示した例が18‐64歳で2倍程度に増え、60歳以上では全く増えなかった。	
100069	2009/12/3	90773	新型インフルエンザ(H1N1)	CDC/MMWR 2009; 58; (Dispatch) 1-3 (2009 April 21)	2009年4月17、米CDCはカリフォルニア南部の小児2例の熱性呼吸器疾患をブタインフルエンザA(H1N1)感染であると特定した。2症例から検出されたウイルスは、アマンダジン、リマンダジンに抵抗性があり、米国やそれ以外の国でも報告されたことがないブタ又はヒトインフルエンザウイルスの遺伝子片を併せ持っており、固有の遺伝子断片の組み合わせが含まれていた。いずれの小児もブタとの接触はなく、感染源は不明である。	
100084	2010/1/26	90859	新型インフルエンザ(H1N1)	CDC/MMWR 2009;58(28);773-778	2009年5月28日、Dallas County Department of Health and Human Services (DCHHS)は5月18‐28日に、ダラス郡(County)内で入院した、新型インフルエンザA感染に関連した神経系の合併症を伴う4例の小児についてCDCに報告した。これまで季節性インフルエンザの気道感染に関連した神経系の合併症は報告されているが、新型インフルエンザに関しては報告がない。患者は7, 10, 11および17歳であり、ILI(influenza-likeillness:インフルエンザ様症状)の症状と痙攣もしくは精神状態の変化のため入院し、3例に脳波に異常が認められた。また、4例すべてに新型インフルエンザA(H1N1)ウイルスRNAが鼻咽頭検査では認められ、脳脊髄液からは認められなかった。4例すべては回復し、神経学的後遺症はなかった。	
100070	2009/12/3	90774	新型インフルエンザ(H1N1)	Eurosurveillance 2009; 14; 19244	2009年5月から6月における日本のインフルエンザA(H1N1)感染に関する疫学的な特徴がまとめられた。日本の16の都道府県から、インフルエンザA型(H1N1)ウイルス確定症例が合計401例報告された。最も感染の多かった2地域は、高校でアウトブレイクが発生し休校に至った大阪市および神戸市であり、6月4日までにこの2県で357例の感染が報告され、64%が15-19歳、10%が10-14歳であり、60歳以上は1%であった。2009年6月4日現在、重症患者および死亡例の報告はなく、インフルエンザA(H1N1)に感染した患者の病状の程度は季節性インフルエンザと同程度であった。	
100070	2009/12/3	90774	新型インフルエンザ(H1N1)	FDA/CBER 2009 April 30	新型インフルエンザ(H1N1)の輸血を介した感染可能性について。輸血により季節性インフルエンザに感染した例はこれまで報告されたことが無く、新型インフルエンザについても報告されていない。現時点で、輸血のメリットは新型インフルエンザの理論的リスクをはるかに上回る。なお、血漿分画製剤については製造工程におけるクリアランスが十分であることが確認されている。	

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100084	2010/1/26	90859	新型インフルエンザ(H1N1)	FDA/CBER Guidance for Industry 2009 November	2009年11月、FDAは企業向けガイダンス案、「パンデミック(H1N1)2009ウイルスに対応した供血者の適合性、血液製剤の安全性および血液供給の保全について評価するための勧告」を発表した。勧告(Recommendation)の内容は、 A. 交代要員の教育 B. 供血者の適格性、供血延期および製品管理 供血者の適格性：原則、供血者の治療歴は採血時に収集されるが、全血もしくは原料血漿用では、供血日に収集すること。 供血延期：パンデミック(H1N1)2009インフルエンザ感染又は疑いのある患者、もしくはインフルエンザ様症状を呈する患者との接触のあった供血者について、利用可能なデータは供血延期を支持していない。供血者が供血日に健康であることを確保するため、パンデミック(H1N1)2009インフルエンザ感染又は疑いのある供血者は、解熱剤の利用なく解熱し、無症状となってから少なくとも24時間の供血延期をすること。パンデミック(H1N1)2009ウイルスに対する生もしくは不活化インフルエンザワクチンを接種した後、もしくは、予防目的で抗ウイルス薬であるオセルタミビルおよびザナミビルを使用した後の供血者について、利用可能なデータは供血延期を支持していない。しかし、パンデミック(H1N1)2009インフルエンザ感染又は疑いのために抗ウイルス薬を服用した供血者は、上述と同様の状態から少なくとも24時間の供血延期をすること。 血液製剤の管理：供血後48時間以内にパンデミック(H1N1)2009の感染又は疑いがある、もしくは、インフルエンザ様症状を呈したという供血後の情報を受けた際には、Medical DirectorはSOP(標準操作手順書)に従い、既に供血された製品の安全性を評価すること。なお、この勧告は、輸血用全血および血液成分の献血に適用される。 C. 承認された申請内容の変更	**14**
100070	2009/12/3	90774	新型インフルエンザ(H1N1)	N Engl J Med 2009; 360; 2605-2615	4月15日から5月5日の間、米国の41州において、総計642症例からヒトにおける新規ブタインフルエンザA(H1N1)ウイルスの感染を確認した。	
100080	2009/12/22	90825	新型インフルエンザ(H1N1)	Sience 2009; 10.1126/SCIENCE.1176062	新型インフルエンザA(H1N1)ウイルスは世界中に急速に広まっている。パンデミックの可能性を判断するのはデータが限られているため難しいが、適切な保険対応を伝えるには必須である。メキシコでの大流行、国際的な広がりの早期情報およびウイルス遺伝的変異について分析することにより、感染力と重症度の早期評価を実施した。	
100080	2009/12/22	90825	新型インフルエンザ(H1N1)	The Canadian Press 2009 September 16	オーストラリアの研究グループは新型A1N1ウイルスに感染し重症となった妊婦では、ウイルスと戦い、体がワクチンに反応する助けとなる、特定の抗体が低値である事を発見した。ICUで治療中のブタインフルエンザ感染患者すべての抗体レベルを個々のサブタイプまで調べた結果、IgG2のレベルが低値であった。妊娠女性についてのみ調べた結果であるが、このIgG2欠損が、ほとんどの人はインフルエンザ症状のみで治癒するが少数例は危篤となる理由が説明できる可能性がある。	
100069	2009/12/3	90773	新型インフルエンザ(H1N1)	厚生労働省 新型インフルエンザに関する報道発表資料 2009 May 16	兵庫県神戸市における新型インフルエンザ(インフルエンザA／H1N1)が疑われる患者発生についての報告。国内最初の新型インフルエンザ患者が確認された。患者は10代後半の男性。本人に渡航歴はない。国立感染症研究所からの検査の結果、A型(＋)、ヒトH1(-)、ヒトH3(-)、新型H1(+)であったため、新型インフルエンザ(インフルエンザA/H1N1)が否定でず、新型インフルエンザが疑われる患者として神戸市に届出があった。患者は感染症法に基づき、神戸市内の感染症指定医療機関に入院した。	

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100070	2009/12/3	90774	BSE	OIE (http://www.oie.int/eng/info/en_esbmonde.htm)	1989年から2008年までに、世界各国（英国を除く）から国際獣疫事務局（OIE）に報告されたBSEの報告数である。	
100070	2009/12/3	90774	BSE	OIE (http://www.oie.int/eng/info/en_esbru.htm.)	1987年以前から2008年までに、英国から国際獣疫事務局（OIE）に報告されたBSEの報告である。	
100080	2009/12/22	90825	BSE	PLoSONE 2009; 4; E6175	魚類におけるTSE(transmissible spongiform encephalopathies)発症についての知見を得るため、gilthead sea beam(sparus aurata:ヨーロッパヘダイ)にBSE感染ウシもしくはスクレイピー感染ヒツジのホモジネートを経口投与した。魚に臨床症状は現れなかったが、投与2年後、魚の脳は神経変性の徴候と抗タイPrP抗体に陽性を示す沈着物の蓄積が認められた。非感染動物由来の脳を投与された対照群はこのような徴候はなかった。TSE感染脳よりもBSE感染脳投与された魚に多数のプロテアーゼK抵抗性沈着物が急速に現れ、アミロイド様成分と一致した。公衆衛生上の潜在的なリスクの懸念が高まる。	15
100099	2010/2/23	90951	変異型クロイツフェルト･ヤコブ病	AABB Weekly Report 15(39) 2009 October 22	米国AABBのTTD(Trsnsfusion Transmitted Diseases) CommitteeがAABBのBoard of Directorへ提出したTSE (Transmissible Spongiform Encephalopathies)の現状と輸血の安全性に関する報告書である。これまで、vCJDを発症した3例からの輸血によってvCJDプリオンが伝播した4例の報告がある。そのうち、3例はvCJD発症に至り、他の要因で亡くなった1例は脾臓およびリンパ節からvCJDプリオンが検出されたが、vCJDの兆候を示さなかった。その患者はプリオン遺伝子の129番目コドンがヘテロ（MV)であった。また、vCJDを発症したドナーから血漿分画製剤を投与された患者に、vCJDプリオンが検出されたが、この患者もvCJDの兆候はなかった。米国FDAは2009年6月に、米国内供給された血漿製剤給血者のvCJD伝播のリスクに関する新しいモデルをTSE Advisory Committeeで発表したが、最大推定リスクは1/12,000のままであり、米国患者のリスクは「極めて低い」としている。しかし、MVもしくはVV遺伝子型である無症候患者から病原性プリオンが検出されたことから、非MM遺伝子型患者にvCJD症状が現れるか、非MM遺伝子型患者はvCJDプリオンの感染キャリアーとなるかについて解決が待たれる。	16
100070	2009/12/3	90774	変異型クロイツフェルト･ヤコブ病	BMJ 2009 May 21	英国イングランドおよびスコットランドで扁桃摘出術により摘出された匿名の扁桃腺検体を対象に、プリオンプロテイン（PrPCJD)に関連した陽性率をcross sectional opportunistic survey (随時横断調査）により調査した。2008年9月末までに63,007の検査を行い、このうち12,753検体は最もvCJDが発症した1961-85年の出生コホート由来であり、19,908検体はBSEに曝露された可能性のある1986-95年コホートから集められた。2種類の酵素免疫法両方に明確に陽性を示す検体は無く、276検体はいずれかの検査に初回陽性を示し、その繰り返し陽性率は15%であった。免疫組織化学法もしくは免疫ブロット法を行った結果、この276検体を含め、陽性を示す検体はなかった。1961-85年の出生コホート由来の扁桃検体におけるPrPCJD陽性率は0/32661であり、1961-85年の出生コホート由来については0であり、過去の虫垂組織の調査結果よりは低かったが、矛盾はなかった。	
100075	2009/12/18	90810	変異型クロイツフェルト･ヤコブ病	Department of Health 2009 June 5	血友病患者の脾臓中に異常プリオン蛋白質が発見されたことを受け、CJD事故委員会の要請により「vCJD Risk Assessment Calculations for a Patient with Multiple Routes of Exposure」報告書がDepartment of Healthによって作成された。感染可能性のある種々の経路を設定し、それぞれの相対的な感染確率を検討した報告である。	

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100079	2009/12/18	90814	変異型クロイツフェルト･ヤコブ病	FDA/CBER 2009 September 7	FDAのCBERは、米国承認血漿由来第VIII因子製品(pdFVIII)によるvCJD(variant Creutzfeldt-Jakob disease)リスクの可能性についての概要を公表し、要点として以下が示された。 ○近年、米国承認pdFVIII製品を投与された血友病AおよびvonWillebrand病患者にvCJDが発病するリスクに関して疑問が提起されている。 ○リスク評価の結果、FDA、CDCおよびNIHも含めて米国PHS(Public Health Service)は、米国承認pdFVIII製品を投与された血友病AおよびvonWillebrand病患者へのvCJDリスクは、はっきりとは分からないが、極めて小さい可能性が最も考えられる。第IX因子を含めた他の血漿由来製品によるvCJDリスクは同程度小さいもしくはより小さい可能性が最も考えられる。 ○新しい情報を得るには、Hemophilia Treatment Centerの血友病もしくはvon Willebrandにおける専門家に尋ねること。	17
100080	2009/12/22	90825	変異型クロイツフェルト･ヤコブ病	FDA/TSE advisary committee 2009 June 16	英国でvCJDに関連した凝固因子製剤を11年前に投与された血友病患者のvCJD感染の報告を受けて、米国におけるリスク管理戦略を再評価した。その結果は、米国で承認されている第VIII因子製剤からのvCJD感染のリスクは極めて低いと考えられるが断言はできない、という従来と同様の評価である。	
100080	2009/12/22	90825	変異型クロイツフェルト･ヤコブ病	HPA 2009 May 22	2004年にHealth Protection Agencyは扁桃腺に蓄積されたvCJD関連プリオンタンパク質の大規模な調査により、無症候性vCJD保有率を検討するNational Anonymous Tissue Archive(NATA)を開始。既に63000例の扁桃腺組織の収集･解析を行っており、100000例まで収集する計画であるが、現在のところ陽性サンプルは一つもなかった。	
100070	2009/12/3	90774	変異型クロイツフェルト･ヤコブ病	ProMED-mail 20090108.0076	英国CJDサーベイランスユニットの統計によると、2009年1月5日時点でvCJD死亡患者数総数には変化はなく167例のままであり、英国におけるvCJD流行は減少しつつあるとする見解に一致する。	
100085	2010/1/27	90881	変異型クロイツフェルト･ヤコブ病	Vox Sanguinis 2009; 96; 270	1995年から3回/週でIVIG治療を受けていた61歳女性は、1997年1月～1998年2月の期間に、後にvCJDを発症した供血者由来の製剤を使用していた。この女性の死亡後、剖検により脾臓、リンパ節、脳内のプリオン蛋白を検査したが、検出されなかった。	
100084	2010/1/26	90859	変異型クロイツフェルト･ヤコブ病	Vox Sanguinis 2009; 97(3); 207-210	英国ではvCJD(variant Creutzfeldt-Jakob disease)症例における血漿分画製剤の投与歴を明らかにするため、英国NCJDSU(National CJD Surveillance Unit)が患者の親戚や診療機関および病院を通して集めた記録の調査が行われた。NCJDSUでは問い合わせのあった全vCJD症例につき、リスク要因となる情報収集を行っている。その結果、168例の英国内vCJD症例のうち9例がのべ12回血漿分画製剤の投与を受けていた(1例はvCJDリスクが起きる前の1970年であり、それ以外は1989-1998年であった)。英国CJD Incident Panelのリスク評価基準によると、11については低リスク製品であり、1つは低もしくは中程度のリスクであった。今日までの英国内vCJD症例はいずれに関しても血漿分画製剤投与による感染ではないと考えられたが、今後、vCJDを発症する可能性は排除されない。	18

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 10. 14	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		紀野修一, 安村敏, 山口一成. 第13回日本肝臓学会大会; 2009 Oct 14-15; 京都.	日本

研究報告の概要

○輸血前後の感染症マーカー検査からみたB型肝炎ウイルス再活性化に関する全国調査結果

【目的】輸血後肝炎は血液製剤が原因と考えられてきたが、肝炎ウイルス再活性化、院内感染なども原因となり得る。厚生労働省通知では輸血前のHBs抗原、HBs抗体、HBc抗体、輸血3ヶ月後にHBV DNAの検査が推奨されている。輸血後検査におけるHBV陽性例の発生状況と原因を全国調査を実施し検討した。

【対象と方法】日本輸血・細胞治療学会が行った「平成19年度輸血業務に関する総合的アンケート調査」の詳細設問に輸血と感染の項目を追加した。2007年1月～12月の輸血後検査におけるHBV DNA又はHBs抗原陽性例経験の有無を問い、有経験施設には個別調査協力を依頼し、HBVマーカーの検査結果、原疾患、抗腫瘍薬・免疫抑制薬使用や輸血後肝炎発症の有無などを調査した。

【成績】300床以上で血液製剤使用量が3000単位以上の全医療機関777を含む1341施設を対象とした。844施設から回答があり、そのうち375施設が詳細設問にも回答した。輸血後HBV陽性例の経験施設は37施設(70症例)で、個別調査協力施設は18施設(37例)であった。原疾患は血液疾患9例、非血液疾患20例、不明8例。抗腫瘍薬は投与あり7例、投与なし13例、未記載17例。免疫抑制薬はそれぞれ4例、16例、17例。分子標的薬はそれぞれ1例、18例、18例であった。輸血後肝炎発症は7例であった。輸血前又は輸血前保管検体の検査成績と献血者保管検体の個別NATの成績から、1)既感染19例、2)輸血感染4例、3)再活性化6例、4)その他(院内感染、性感染など)0例、5)分類不能8例に分けられた。

【結語】輸血を要する治療を行った患者において、HBV再活性化は少なからず存在することが判明した。輸血によるHBV伝播とHBV再活性化の鑑別のためには、輸血前のHBs抗体・HBc抗体検査か、輸血前検体保管が必要である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
日本輸血・細胞治療学会が行ったアンケート調査から、輸血を要する治療を行った患者において血液製剤が原因ではないHBV再活性化が少なからず存在することが判明したとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、薬事法及び関連法令に従い輸血副作用・感染症情報を収集し、医薬品医療機器総合機構を通じて国に報告しているほか、「血液製剤等に係る遡及調査ガイドライン」(平成20年12月26日付薬食発第1226011号)に基づき、輸血感染症の調査を行っている。輸血感染症に関する新たな知見等について今後も情報の収集に努める。検査精度向上のため、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。

肝S3-12　HBc抗体陽性者からのde novo B型肝炎の臨床像 —肝移植例と化学療法施行例の比較検討—

京都大大学院・消化器内科学
○上田　佳秀，丸澤　宏之，千葉　勉

【目的】HBs抗原陰性でもHBc抗体陽性を示す健常人の肝臓内には微量ながらB型肝炎ウイルス(HBV)が存在し、免疫抑制状態ではその潜伏HBVが活性化することが、肝移植症例の知見から明らかとなっている。今回、肝移植症例におけるHBV活性化の全体像を明らかにし、非肝移植例のde novo B型肝炎との相違点を検討することにより、その病態ならびに治療法を考察することを目的とした。【方法】京都大学において、HBc抗体陽性ドナーからの肝移植後にHBVの活性化を認めた36例、ならびに、血液疾患や膠原病に対する治療後にde novo B型肝炎の発症を認めた7例について、その臨床像と治療経過について解析した。【成績】HBc抗体陽性ドナーからの肝移植後に予防策を行わなかった17例では全例で、HBIGの予防投与を行った75例においては19例(25%)でHBV活性化を認めた。HBIG投与下での活性化の原因として、HBs抗体エスケープ変異株の出現、免疫抑制剤の増量、HBIGの投与中断の3つが明らかとなった。HBV活性化を認めた36例中、活性化早期に治療を行わなかった22例においては、17例(77%)がB型慢性肝炎へと移行、1例が急性肝不全で死亡した。一方、HBV活性化早期に核酸アナログ製剤の投与を行った14症例中13例(93%)でHBs抗原の陰性化を認めた。非肝移植症例においてde novo B型肝炎の発症を認めた症例は、6例が血液疾患治療後、1例はSLEに対するステロイド治療後であった。核酸アナログを投与しなかった4例では2例が慢性肝炎へと移行し、1例が急性肝不全で死亡したのに対し、活性化早期に核酸アナログ投与を行った症例3例はいずれもHBs抗原が陰性化し、その後核酸アナログを中止してもHBV陰性が持続した。【結論】HBc抗体陽性ドナーからの肝移植後のHBV活性化症例から、HBV既感染者の病態、活性化後の自然経過、治療法が明らかとなった。これらの知見は、非肝移植例で生じるde novo B型肝炎の診療に応用することが可能であり、HBV活性化例を早期に発見して核酸アナログ製剤を投与することが重要である。

B型肝炎　　肝移植

肝S3-13　輸血前後の感染症マーカー検査からみたB型肝炎ウイルス再活性化に関する全国調査結果

旭川医大病院・臨床検査・輸血部[1]，富山大附属病院・輸血・細胞治療部[2]，国立感染症研究所・血液・安全性研究部[3]
○紀野　修一[1]，安村　敏[2]，山口　一成[3]

【目的】輸血後肝炎は血液製剤が原因と考えられてきたが、肝炎ウイルス再活性化、院内感染なども原因となり得る。厚生労働省通知では輸血前にHBs抗原、HBs抗体、HBc抗体を、輸血3ヶ月後にHBVDNAを検査することが推奨されている。本研究では輸血後検査におけるHBV陽性例の発生状況とその原因を全国調査を実施し検討した。【対象と方法】日本輸血・細胞治療学会が行った「平成19年度輸血業務に関する総合的アンケート調査」の詳細設問に輸血と感染の項目を追加した。2007年1月～12月の輸血後検査におけるHBVDNA又はHBs抗原陽性例経験の有無を問い、有経験施設には個別調査協力を依頼した。対象施設に個別調査票を送付し、HBVマーカーの検査結果、原疾患、抗腫瘍薬・免疫抑制薬使用や輸血後肝炎発症の有無などを調査した。【成績】アンケート調査は300床以上で血液製剤使用量が3000単位以上の全医療機関777を含む1341施設を対象に実施した。844施設から回答があり、そのうち375施設が詳細設問にも回答した。輸血後HBV陽性例の経験施設は37施設(70症例)で、個別調査協力施設は18施設(37例)であった。原疾患は血液疾患9例、非血液疾患20例、不明8例。抗腫瘍薬は投与あり7例、投与なし13例、未記載17例。免疫抑制薬はそれぞれ4例、16例、17例。分子標的薬はそれぞれ1例、18例、18例であった。輸血後肝炎発症は7例であった。輸血前又は輸血前保管検体の検査成績と、献血者保管検体の個別NAT検査の成績を元に、1)既感染例、2)輸血感染例、3)再活性化例、4)その他（院内感染、性感染など）、5)分類不能に分けた。各分類に該当する症例はそれぞれ19例、4例、6例、0例、8例であった。【結語】輸血を要する治療を行った患者において、HBV再活性化は少なからず存在することが判明した。輸血によるHBV伝播とHBV再活性化の鑑別のためには、輸血前のHBs抗体・HBc抗体検査か、輸血前検体保管が必要である。

B型肝炎ウイルスの再活性化　輸血前後の感染症検査

別紙様式第2-1

No. 11

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 9. 16	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		FDA, CBER. Available from: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm180817.htm	米国

研究報告の概要

○業界向けガイダンス 人免疫不全ウイルス1型(HIV-1)グループO感染リスクの高いドナー取扱いにかかる勧告
このガイダンスは即時適用すること。
A. HIV-1グループO感染リスクの高い供血者を排除するため、問診事項にハイリスク行動を含む直接的な質問を含めること。
1.1977年以降、以下の国で生まれたか、居住していたことがありますか。それはいつですか。
カメルーン、ベナン、中央アフリカ、チャド、コンゴ、赤道ギニア、ケニア、ガボン、ニジェール、ナイジェリア、セネガル、トーゴ、ザンビア
2.1977年以降、上記国への渡航歴がある場合、輸血や血液製剤を使用した治療を受けましたか。それはいつですか。
3.1977年以降に上記国で生まれた人もしくは居住していた人と性的接触を持ちましたか。それはいつですか。
これらの質問に「はい」と答えた供血者の延期期間については永久としないことを推奨する。供血延期となった供血者は、最後のHIV-1グループOへの曝露機会から1年の保留期間をおいて、上記質問の回答内容及び下記Cの勧告に基づきリエントリーを考慮してもよい。
B. HIV-1グループO検出のために認可された抗HIV1/2のスクリーニング試薬を使用する場合、上記の問診を中止してもよい。
C. HIV-1グループO感染リスク関連の問診事項に、以前もしくは最近「はい」と答えて供血延期とされた供血者については、最後のHIV-1グループOへの曝露から1年の保留期間をおいて以下の条件を満たせば、供血適格者としてリエントリーしてもよい。
1.当該供血者の現在の供血時に抗HIV1/2のグループOが検出できる検査が行われ、陰性となった場合。
2.当該供血者が他の全ての適格性条件を満たした場合。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国食品医薬品局が、HIV-1グループO感染リスクの高いドナーの取扱いにかかる勧告を発表したとの報告である。	日本赤十字社では、HIV抗体検査にこれまでの凝集法と比べてより感度の高い化学発光酵素免疫測定法(CLEIA)を導入したことに加え、20プールNATについてもHIV-2及びHIVグループOの検出が可能な新NATシステムを導入し、陽性血液を排除している。国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。

(2)

Guidance for Industry

Recommendations for Management of Donors at Increased Risk for Human Immunodeficiency Virus Type 1 (HIV-1) Group O Infection

This guidance is for immediate implementation.

FDA is issuing this guidance for immediate implementation in accordance with 21 CFR 10.115(g)(4)(i). Submit written comments on this guidance at any time to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. You should identify all comments with the title of this guidance.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
August 2009

Contains Nonbinding Recommendations

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Contains Nonbinding Recommendations

Guidance for Industry

Recommendations for Management of Donors at Increased Risk for Human Immunodeficiency Virus Type 1 (HIV-1) Group O Infection

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, the Food and Drug Administration (FDA), are providing you, blood and plasma establishments, with a revised list of countries that should be included in questions for identifying donors at increased risk for HIV-1 group O infection. We are also providing you with recommendations for discontinuing the use of some questions used to identify these donors and for management of donors previously deferred.

This guidance supersedes the memorandum entitled "Interim Recommendations for Deferral of Donors at Increased Risk for HIV-1 Group O Infection," dated December 11, 1996 (Ref. 1). That memorandum contained interim measures to reduce the risk of HIV-1 group O transmission by blood and blood products pending licensure of test kits specifically labeled for detection of antibodies to HIV-1 group O viruses. Now that an FDA-licensed test for detection of antibodies to HIV-1 group O viruses is available, those interim recommendations are no longer current.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The first report confirming the identification of HIV-1 group O in patients from Central and West Africa was published in 1994 (Ref. 2). That same year, the Centers for Disease Control and Prevention (CDC) reported findings of a study indicating that several FDA-licensed HIV antibody screening tests were unable to detect one or two of eight group O sera (Ref. 3). Tests based on recombinant antigens and synthetic peptide antigens failed to detect at least one specimen, whereas three of the five tests based on whole virus lysate antigens detected all specimens.

In June 1994, in response to the inability of some FDA-licensed HIV antibody assays to detect HIV-1 group O sera, FDA Blood Products Advisory Committee (BPAC) recommended that manufacturers modify their test kits to include detection of HIV-1 group O in clinical specimens. Since then, we have requested manufacturers of HIV-1 assays to include group O specific antigens or sequences in their test kits to detect antibodies to HIV-1 group O or HIV-1 group O nucleic acid. We also requested that manufacturers test HIV-1 group O specimens in their clinical trials to obtain a specific claim of sensitivity for group O for their test. These requests were initially conveyed to manufacturers as letters to their Investigational New Drug Applications (INDs) and Product License Applications (PLAs), currently referred to as Biologics License Applications (BLAs).

In 1996, FDA issued the memorandum "Interim Recommendations for Deferral of Donors at Increased Risk for HIV-1 Group O Infection," to reduce the risk of HIV-1 group O transmission through blood and blood products. In that memorandum we recommended the inclusion of questions related to HIV-1 group O risk in the donor history questionnaire pending the licensure of test kits specifically labeled for detection of antibodies to HIV-1 group O viruses. These direct questions inquire as to whether the donor was born or resides in specific West and Central African countries where HIV-1 group O is prevalent, or had a history of travel to these countries, a history of blood transfusion or medical treatment since 1977 in these countries, or sexual contact since 1977 with anyone who was born or lived in these countries. We also recommended in the 1996 memorandum that donors who gave an affirmative response to one or more of the questions in the donor history questionnaire related to HIV-1 group O risk be indefinitely deferred pending licensure of test kits specifically labeled for detection of HIV-1 group O.

Since the identification of the first two HIV-1 group O cases in the United States (U.S.) around 1996 (Refs. 4, 5) there have been no additional group O cases that have been conclusively identified in the U.S. HIV-1 group O infection in the U.S. continues to be extremely rare.

In August 2003, FDA approved the biologics license application for the first donor screening test specifically labeled as sensitive for detection of antibodies to HIV-1 group O, the Genetic Systems™ HIV-1/HIV-2 *Plus O* EIA. Blood and plasma establishments, manufacturers and testing laboratories that are implementing a licensed test that is sensitive for HIV-1 group O antibody detection may use this test to screen blood and plasma donations for antibodies to HIV-1 and HIV-2, including HIV-1 group O. Other assays for HIV-1 group O detection are under development and if approved by FDA, may be used when available for screening blood and plasma donations.

The list of countries included in the interim recommendations in the 1996 memorandum included Cameroon and countries adjacent to Cameroon (Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger, and Nigeria). However, subsequent reports indicated the presence of HIV-1 group O in countries in Africa that are not adjacent to Cameroon, including Senegal, Togo, Zambia, Benin, and Kenya (Refs. 6-8). In addition, the country formerly named Zaire, recently renamed Democratic Republic of Congo, has not identified any cases of HIV-1 group O infections thus far, but the name of the country might be confused with the country of

Congo where HIV-1 group O has been identified. As a result, we recommend revisions to the list of countries of origin or residence where HIV-1 group O is endemic that is used to identify potential donors who are at increased risk of group O infection (see Figure 1 and Table 1).

With the availability of a licensed donor screening test that is sensitive for antibodies to HIV-1 group O, donors who were previously deferred may be eligible for reentry after a waiting period of one year and may be reentered if a current donation from the donor is found to be non-reactive using a group O sensitive anti-HIV-1/2 test.

III. RECOMMENDATIONS

A. Revised Questions to Identify Donors at Increased Risk of HIV-1 Group O Infection

We recommend that the following questions be included in the direct questions on high risk behavior in the donor history questionnaire[1] to exclude donors who are at increased risk for HIV-1 group O infection:

1. Were you born in or have you lived in any of the following countries since 1977: Cameroon, Benin, Central African Republic, Chad, Congo, Equatorial Guinea, Kenya, Gabon, Niger, Nigeria, Senegal, Togo, or Zambia? If so, when?

2. If you have traveled to any of those countries since 1977, did you receive a blood transfusion or any medical treatment with a product made from blood? If so, when?

3. Have you had sexual contact with anyone who was born in or lived in these countries since 1977? If so, when?

We recommend that you defer indefinitely a potential donor who gives an affirmative answer to any of these questions. Donors deferred on this basis (or previously deferred consistent with FDA's 1996 memorandum on "Interim Recommendations for Deferral of Donors at Increased Risk for HIV-1 Group O Infection") may be considered for reentry one year after their last potential exposure to HIV-1 Group O as determined by their responses to the above donor questions, in accordance with the recommendations below in III.C.

B. Discontinuation of the Questions that Address HIV-1 Group O Risk

If you implement a licensed anti-HIV-1/2 test for donor screening that is specifically labeled in the "Intended Use" section of the package insert as sensitive for detection of HIV-1 group O antibodies, you may discontinue use of the questions in section III. A.

[1] Establishments that have implemented the AABB (formerly known as the American Association of Blood Banks) full-length donor history questionnaire (DHQ) and accompanying materials should question donors concerning possible exposure to HIV-1 group O virus using the capture question approach developed for this protocol.

that address HIV-1 group O risk. If you hold a biologics license, you must report this minor change in an annual report (section 601.12(d) of Title 21 Code of Federal Regulations (21 CFR 601.12(d)).

FDA recognizes that by implementing these measures, the safety benefit of the deferral due to potential group O risk is being replaced by the greater safety benefit of the group O sensitive test in a setting of low overall risk to blood safety. We believe these measures are warranted given the rarity of Group O infections in the U.S. as indicated by CDC data (Refs. 4, 5). The risk for individuals to acquire HIV-1 group O by sexual exposures in the U.S. appears to be remote given the rarity of HIV-1 group O in this country.

C. Reentry of Donors Deferred on the Basis of a Response to HIV-1 Group O Risk Question(s)

A donor who was deferred because of a previous or current affirmative response to one or more of the questions in the donor history questionnaire related to HIV-1 group O risk may be eligible for reentry after a waiting period of at least one year following the date of the donor's last potential exposure to HIV-1 group O. The donor may be reentered if:

1. a current donation from the donor is tested and found non-reactive using an anti-HIV-1/2 screening test that is specifically labeled in the "Intended Use" section of the package insert as sensitive for detection of HIV-1 group O antibodies, and

2. the donor meets all other donor eligibility criteria[2].

[2] Since individuals who travel to an area considered endemic for malaria by the Malaria Branch, CDC, are deferred from donating Whole Blood and blood components for one year following their departure from the endemic area, and since the areas considered endemic for malaria include the countries in which HIV-1 group O has been identified, the current deferral for donors potentially exposed to malaria includes donors potentially exposed to HIV-1 group O while in Africa.

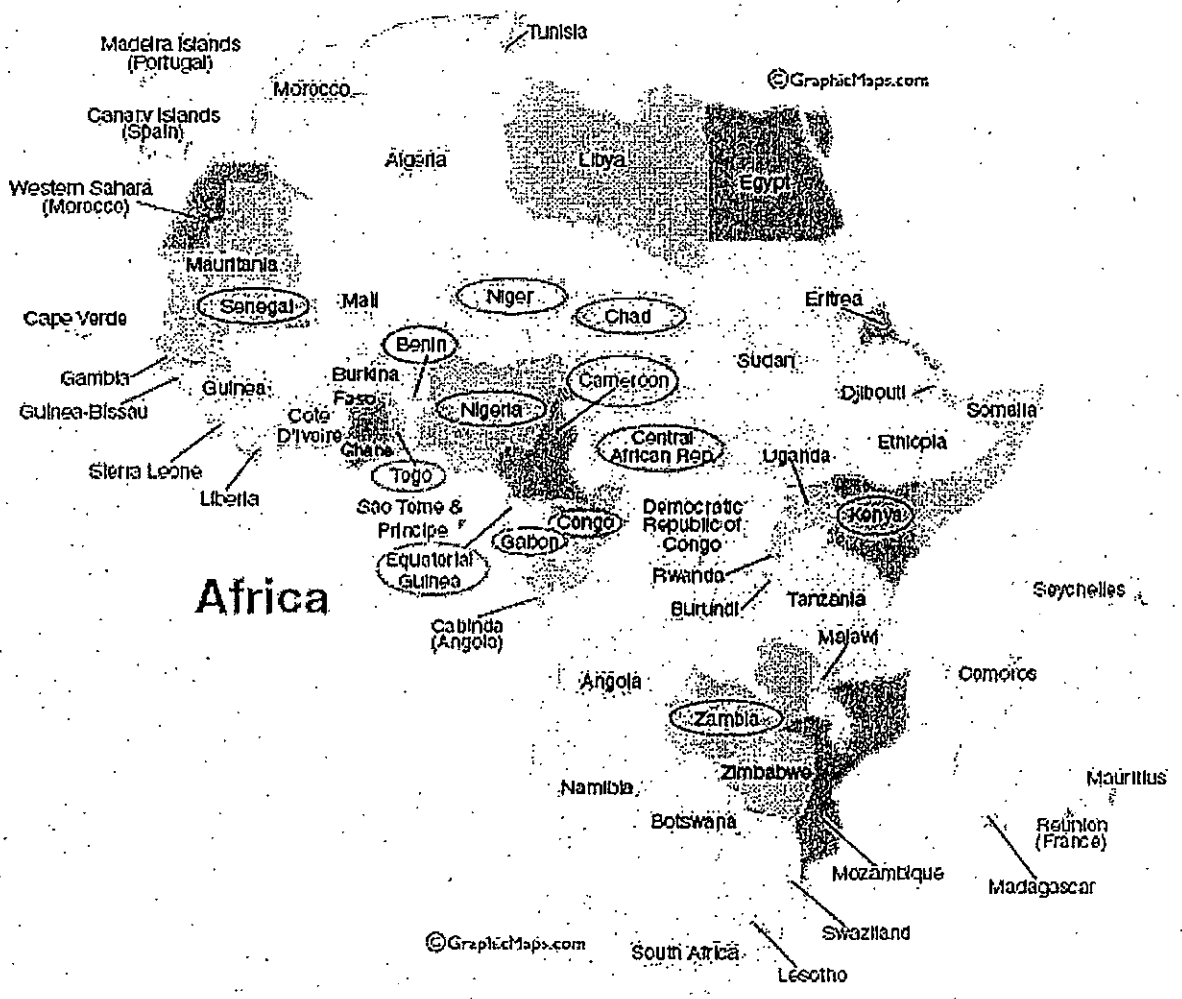

Figure 1. Circled countries are those in which HIV-1 Group O is currently endemic

Table 1. Current and Previous List of Countries with HIV-1 Group O Risk

Current List of Countries	Previous List of Countries
Cameroon	Cameroon
Central African Republic	Central African Republic
Chad	Chad
Congo	Congo
Equatorial Guinea	Equatorial Guinea
Gabon	Gabon
Niger	Niger
Nigeria	Nigeria
Senegal	
Togo	
Zambia	
Benin	
Kenya	

IV. REFERENCES

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医薬品 研究報告 調査報告書

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一般的名称	人赤血球濃厚液	研究報告の公表状況	三浦左千夫, 竹内勤. 第58回日本感染症学会東日本地方会学術集会・第56回日本化学療法学会東日本支部総会合同学会; 2009 Oct 30-31; 東京.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○在日ラテンアメリカ人の慢性シャーガス病キャリアーと2次感染予防

我が国の在日ラテンアメリカ人は既に40万人に達する勢いで増加している。そのうちブラジルからの滞在者が80%を占めており、その8万人が既に定住永住権を取得している。こうした中で、南米特有の風土病シャーガス病患者も散見されるようになった。近年各地医療機関から依頼のあった心疾患患者41名についてシャーガス病病原体 *Trypanosoma Cruzi* (*T. cruzi*) 血清抗体検査を行った。その結果15名 (36.58%) が明らかに陽性と判定され、シャーガス病が示唆された。更に、抗体陽性者について血液を材料にしたPCRを行った結果4名に *T. cruzi*-DNA産物を検出した。病原体の血液内生残が強く示唆されたので、更に血液培養を試みた結果2名 (抗体陽性者の13.3%) から *T. cruzi* 虫体を分離することが出来た。即ち慢性の病原体キャリアーが日本に現存することが明らかとなった。ECGでは不整脈、心エコーで拡張型心筋症を示した。ブラジル、ボリビアの生活歴がある者に関しては、我が国では臨床経験の少ないシャーガス病感染を検討すべきである。一方、消化器系の症状を訴える患者の検査依頼は皆無であったが、心室拡張症で通院している同一患者は消化器症状 (飲み込み困難、排便困難) をも訴えているものの、検査を受けていない。本疾患の特徴は感染者の70%は病型が定まらない慢性感染で、一見健常者とみえることである。本人、家族もその感染を認知するものは少ない。媒介昆虫の存在しない日本国内で感染が起こるとすれば、それは輸血感染、臓器移植による2次感染であると思われる。肝要な点は、事前の抗体チェックでこのような2次感染が防げることである。ラテンアメリカ人の多くを抱える地方自治体は健康保健支援環境を整備し、シャーガス病の2次感染を阻止すべく啓蒙監視活動を強化すべきであり、全国的に行われている善意の献血現場で抗体スクリーニングを実施すべく、体制の整備を行う必要がある。

報告企業の意見	今後の対応
近年各地の医療機関から依頼のあった在日ラテンアメリカ人心疾患患者41名のうち、15名が *Trypanosoma Cruzi* 抗体陽性と判定され、PCRで4名の血液中からDNAを検出、2名から虫体が分離されたとの報告である。	日本赤十字社は、輸血感染症対策としてシャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

041

在日ラテンアメリカ人の慢性シャーガス病キャリアーと2次感染予防

慶應義塾大学医学部熱帯医学寄生虫学

○三浦 左千夫，竹内 勤

我が国の在日ラテンアメリカ人は既に40万人に達する勢いで増加している。そのうちブラジルからの滞在者が80%を占めており、その8万人が既に定住永住権を取得している。こうした中で、南米特有の風土病シャーガス病患者も散見されるようになった。近年各地医療機関から依頼のあった心疾患患者41名についてシャーガス病病原体*Trypanosoma cruzi (T.cruzi)* 血清抗体検査を行った。その結果15名 (36.58%) が明らかに陽性と判定され、シャーガス病が示唆された。更に、抗体陽性者について血液を材料にしたPCRを行った結果4名に*T.cruzi*－DNA産物を検出した。病原体の血液内生残が強く示唆されたので、更に血液培養を試みた結果2名 (抗体陽性者の13.3%) から*T.cruzi*虫体を分離することが出来た。即ち慢性の病原体キャリアーが日本に現存することが明らかとなった。ECGでは不整脈、心エコーで拡張型心筋症を示した。ブラジル、ボリビアの生活歴がある者に関しては、我が国では臨床経験の少ないシャーガス病感染を検討すべきである。

一方、消化器系の症状を訴える患者の検査依頼は皆無であったが、心室拡張症で通院している同一患者は消化器症状 (飲み込み困難、排便困難) をも訴えているものの、検査を受けていない。

本疾患の特徴は感染者の70%は病型が定まらない慢性感染で、一見健常者とみえることである。本人、家族もその感染を認知するものは少ない。

媒介昆虫の存在しない日本国内で感染が起こるとすれば、それは輸血感染、臓器移植による2次感染であると思われる。肝要な点は、事前の抗体チェックでこのような2次感染が防げることである。ラテンアメリカ人の多くを抱える地方自治体は健康保健支援環境を整備し、シャーガス病の2次感染を阻止すべく啓蒙監視活動を強化すべきであり、全国的に行われている善意の献血現場で抗体スクリーニングを実施すべく、体制の整備を行う必要がある。

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遷延する関節痛を主訴に来院したチクングニヤ熱の3例

[1]国立国際医療センター戸山病院 国際疾病センター，[2]長崎大学熱帯医学研究所 臨床医学分野，[3]国立感染症研究所 ウイルス第一部

○水野 泰孝[1]，氏家 無限[1,2]，竹下 望[1]，加藤 康幸[1]，金川 修造[1]，工藤 宏一郎[1]，林 昌宏[3]，高崎 智彦[3]

チクングニヤ熱(Chikungunya fever; CHIKF) は発熱、関節炎、発疹を主症状とする熱性疾患であり、臨床症状や検査所見はデング熱に類似するが、遷延する関節症状が特徴的である。本年になり東南アジア地域を中心に再びCHIKF流行が拡大しており、当センターにおいても2009年5月から6月にかけての2ヶ月間で、東南アジアから帰国後に遷延する関節痛を主訴に来院した3例を血清学的にCHIKFと診断したのでその概要を報告する。(症例1) 52歳日本人男性。2009年3月26日から4月5日までインドネシア・スマトラ島へ蝶の採集目的で滞在。3月31日に39.5度の発熱、関節痛 (両手足首、両膝) が出現。翌日には解熱したものの関節痛は持続したため、帰国後5月上旬に近医整形外科受診。関節リウマチ、痛風検査を実施されるも陰性であったため、精査目的で当センターを受診した。(症例2) 30歳日本人男性。2009年4月16日より6月14日までインドネシア・ジャワ島へ舞台公演目的で滞在。5月13日に発熱、関節痛 (右足首、左膝、右肩)、頭痛、発疹が出現。4日後に解熱したものの関節痛は持続したため6月22日に当センターを受診した。(症例3) 39歳日本人女性。2009年4月4日より6月28日までマレーシア・クアラルンプール郊外に帯同家族として滞在。5月12日に39.5度の発熱、関節痛 (両手足首)、発疹、歯肉炎が出現。現地の病院で膠原病スクリーニング等の精査を受け、異常所見は認められなかったものの関節痛が持続するため、6月30日に当センターを受診した。いずれの症例も来院時の検査でチクングニヤウイルスIgM抗体及び中和抗体陽性であり、血清学的にCHIKFと確定診断した。流行地から帰国した後、遷延する関節症状を訴える患者を診療する場合には、リウマチ性疾患との鑑別の上でもCHIKFの可能性を考慮に入れた正確な血清診断を行うべきである。

医薬品 研究報告 調査報告書

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一般的名称	人赤血球濃厚液	研究報告の公表状況	Holzmann H, Aberle SW, Stiasny K, Werner P, Mischak A, Zainer B, et al. Emerg Infect Dis. 2009 Oct. Available from http://www.cdc.gov/EID/content/15/10/1672.htm	公表国 オーストリア
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			

研究報告の概要

⊙オーストリア山岳地域におけるヤギ・チーズ摂食によるダニ媒介性脳炎
オーストリアの海抜1,500m域の山岳地域において、2008年7月に、ヒト6名とブタ4頭に非殺菌ヤギ乳を介して伝播したダニ媒介性脳炎ウイルス(TBEV)を報告する。
初発患者は43歳の羊飼いで、高山牧草地に24日間滞在した後、非細菌性尿道炎とインフルエンザ様症状で入院し髄膜炎の臨床症状を発症した。ELISAによって血清学的にTBEV感染陽性と確認された。患者はダニに咬まれた記憶はなかったが、発症8～11日前に非殺菌ヤギ乳及び牛乳から製造された自家製チーズを食べていた。さらに調査したところ、同じチーズを食べた6名中5名が血清学的にTBEV感染と診断された。
チーズ原料の乳を搾ったヤギはHI及び中和抗体検査でTBEV陽性と確認された。ウシ3頭は抗体陰性であり、問題のチーズは検査用に入手できなかった。牧草地で飼育されていたブタ4頭がホエイとヤギ乳を与えられており、TBEV抗体陽性となったが、臨床症状は見られなかった。
このアウトブレイクは、中央ヨーロッパ高地におけるTBEVの新興と、TBEVの経口感染の高い効率性を示している。感染した6名については、ワクチンを接種していれば感染を予防できただろう。

報告企業の意見	今後の対応
オーストリア山岳地域において、ヒト6名とブタ4頭に非殺菌ヤギ乳を介してダニ媒介性脳炎ウイルスが伝播したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

④

Tick-borne Encephalitis from Eating Goat Cheese in a Mountain Region of Austria

Heidemarie Holzmann, Stephan W. Aberle, Karin Stiasny, Philipp Werner, Andreas Mischak, Bernhard Zainer, Markus Netzer, Stefan Koppi, Elmar Bechter, and Franz X. Heinz

We report transmission of tick-borne encephalitis virus (TBEV) in July 2008 through nonpasteurized goat milk to 6 humans and 4 domestic pigs in an alpine pasture 1,500 m above sea level. This outbreak indicates the emergence of ticks and TBEV at increasing altitudes in central Europe and the efficiency of oral transmission of TBEV.

Tick-borne encephalitis virus (TBEV) is a human pathogenic flavivirus that is endemic to many European countries and to parts of central and eastern Asia (*1*). Even though vaccination can effectively prevent TBE (*2*), >10,000 cases are reported annually for hospitalized persons in areas of Europe and Asia to which TBE is endemic. TBEV occurs in natural foci characterized by ecologic habitats favorable for ticks, especially in wooded areas within the 7°C isotherm (*3*). The major route of virus transmission is tick bites, but TBEV also can be transmitted during consumption of nonpasteurized milk and milk products from infected animals, primarily goats (*3*). Outbreaks resulting from oral virus transmission are rare in central Europe but more common in eastern Europe and the Baltic states (*3*). Our investigation of TBEV transmitted by milk from a goat in an alpine pasture in a mountainous region provides evidence for a changing TBEV epidemiology in central Europe and the expansion of ticks and TBEV to higher regions.

The Study

We investigated a TBE outbreak, comprising 6 cases, in a mountain region in western Austria in July 2008. The index case occurred in a 43-year-old shepherd who had stayed for 24 days at his alpine pasture (1,564 m above sea level) before he was hospitalized for nonbacterial urethritis and nonspecific influenza-like symptoms (including pain in the lower abdomen and legs), followed by clinical signs of meningitis. TBEV infection was confirmed serologically by ELISA demonstration of specific immunoglobulin (Ig) M and IgG in serum and cerebrospinal fluid. The patient did not remember a tick bite but had eaten self-made cheese prepared from a mixture of nonpasteurized goat milk and cow milk 8–11 days before illness onset; further investigation found 6 additional persons who had eaten the same cheese (Figure). For 5 of them, recent TBEV infection was serologically proven (Table). For 3 of these persons (2 men, 44 and 65 years of age; and 1 woman, 60 years of age), similar to the index patient, a typical biphasic course and symptoms of TBE (nonspecific flu-like symptoms followed by fever, cephalea, meningism, and ataxia after 4–10 days) developed and they were hospitalized. The 2 other persons who had eaten the cheese (female, 37 and 7 years of age) were clinically asymptomatic. The noninfected person had vomited shortly after eating the cheese because of a gastric banding. None of the infected persons had been vaccinated against TBEV.

The cheese was prepared from a mixture of fresh milk from 1 goat and 3 cows and was eaten shortly after production. Detection of TBEV-specific hemagglutination inhibiting (HI) and neutralizing antibodies in the goat's serum proved infection in the goat; the 3 cows were seronegative for TBEV. At the time of this investigation (1 month after cheese production), TBEV was already undetectable by PCR in serum and milk of the goat. Cheese from the 3 batches produced after the contaminated batch was TBEV negative by PCR. The original cheese was no longer available for testing.

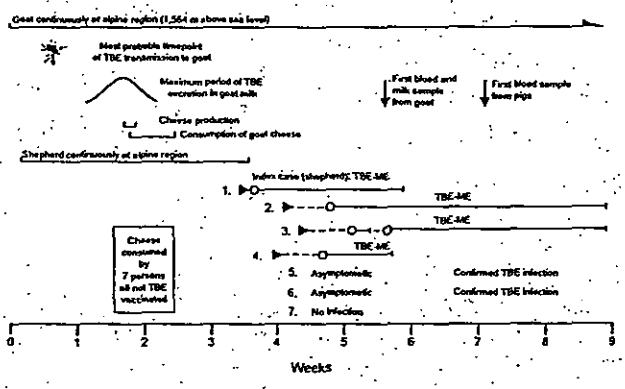

Figure. Time course and series of events of a tick-borne encephalitis (TBE) outbreak from cheese made with goat milk. Week 0, transport of goat to high altitude; ▶, onset of disease; O—I, hospitalization period; TBEV, tick-borne encephalitis virus; ME, meningoencephalitis.

Author affiliations: Medical University of Vienna, Vienna, Austria (H. Holzmann, S.W. Aberle, K. Stiasny, F.X. Heinz); Regional Hospital, Rankweil, Austria (P. Werner, S. Koppi); and Austrian Public Health Authorities, Vorarlberg, Austria (A. Mischak, B. Zainer, M. Netzer, E. Bechter)

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Table. Infection parameters of 7 persons exposed to TBEV by eating nonpasteurized goat cheese, Austria, 2008*

Sex/ age, y	Incubation, d	Symptoms/signs	Diagnosis	Hospitalized, d	Virologic parameters				TBEV infection confirmed
					Material	TBEV ELISA IgM	TBEV ELISA IgG	TBEV NT	
M/43	11	Fever, cephalea, meningism, aseptic urethritis; CSF: pleocytosis	ME	18	Serum CSF	Pos Bor	Pos Pos	Pos	Yes
M/65	10	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	30	Serum CSF	Pos Bor	Pos Bor	Pos	Yes
F/60	14	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	25	Serum CSF	Pos Pos	Pos Pos	Pos	Yes
M/44	9	Fever, cephalea, meningism, vertigo, cerebellar ataxia; CSF: pleocytosis	ME	9	Serum CSF	Pos Pos	Pos Bor	Pos	Yes
F/37	NA	None	NA	0	Serum	Pos	Pos	Pos	Yes
F/7	NA	None	NA	0	Serum	Pos	Pos	Pos	Yes
F/45	NA	None	NA	0	Serum	Neg	Neg	Neg	No

*TBEV, tick-borne encephalitis virus; NT, neutralization test; CSF, cerebrospinal fluid; Ig, immunoglobulin; ME, meningioencephalitis; pos, positive; bor, borderline; NA, not applicable; neg, negative.

The 4 domestic pigs kept at the alpine pasture and fed with the whey and goat milk, however, were seropositive (TBEV HI- and neutralizing antibodies detected), which indicated TBEV infection, but no clinical signs were observed. Infection with TBEV has been reported in wild boars (*4,5*). Serum samples from 105 goats from pastures in the neighborhood also were investigated for TBEV-specific antibodies; all goats were seronegative.

Conclusions

Our analyses showed that the 6 humans and the 4 pigs were infected through the milk of 1 goat, which had been transported by car from a TBE–nonendemic valley to the alp 12 days before production of the TBEV-contaminated cheese. Experiments have demonstrated that infected domestic animals (i.e., goats, sheep, and cows) can excrete TBEV into milk for ≈3–7 days, beginning as early as the second or third day postinfection (*6–9*). In addition, although cheese was produced once or twice each week, only this ≈1-kg batch of cheese transmitted TBEV. Therefore, all the evidence indicates that the goat was infected at the alpine pasture at an altitude of 1,564 m. Indeed, some ticks were collected from cows that had stayed at this altitude during the entire summer. Analyses of these ticks for TBEV by PCR, however, yielded only negative results.

Our findings provide further evidence for the expansion of TBEV-endemic regions to higher altitudes in central Europe. For example, longitudinal studies in the Czech Republic, a country with similar climatic and ecologic conditions to those of Austria, showed a shift in *Ixodes ricinus* ticks and TBEV, from 700 m in 1981–1983 to 1,100 m altitude in 2001–2005 (*10,11*). Likewise, Zeman and Benes demonstrated that the maximum altitude at which TBEV is found in the Czech Republic gradually moved upward during 1970–2000, corresponding to the rise in temperature during the same period (*12*). In Scandinavia, a northward extension of the geographic range of *I. ricinus* ticks and TBEV since the mid-1980s has also been recognized (*1,13–15*). Climatic changes most likely are the major driving forces for the geographic changes in the distribution of TBEV and its main vector, *I. ricinus*, in Europe.

This report also emphasizes the efficiency of oral transmission of TBEV to humans and to pigs. Six of the 7 persons who ate the cheese and all 4 pigs fed residual milk or whey from the same cheese became infected. Given the excellent effectiveness of the TBE vaccine (*2*), vaccination probably could have prevented all 6 human cases.

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Dr Holzmann is a virologist at the Clinical Institute of Virology, Medical University of Vienna, Austria. Her research interests focus on flaviviruses, hepatitis C virus, and antiviral vaccines.

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Address for correspondence: Heidemarie Holzmann, Clinical Institute of Virology, Medical University of Vienna, Kinderspitalgasse 15, A-1095, Vienna, Austria; email: heidemarie.holzmann@meduniwien.ac.at

医薬品 研究報告 調査報告書

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一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		Bondre VP, Sapkal GN, Yergolkar PN, Fulmali PV, Sankararaman V, Ayachit VM, Mishra AC, Gore MM. J Gen Virol. 2009 Nov;90(Pt 11):2644-9.	インド

研究報告の概要

○インドで分離されたバガザウイルス(BAGV)の遺伝子学的特性と脳炎患者から採取した血清中抗BAGV抗体のエビデンス
1996年のインドのケララ州における脳炎アウトブレイクの調査時、コガタアカイエカのプールからアルボウイルスが分離された。補体結合検査により、日本脳炎とウエストナイルウイルスに交差反応を起こす可能性のあるアルボウイルスの特徴が明らかとなった。プラークを精製したアルボウイルス分離株に対する過免疫血清を使用し、プラーク減少／中和検査を行った。血清は日本脳炎ウイルスで陽性を示さず、ウエストナイルウイルスで弱陽性であった。全ORF配列解析で、当該アルボウイルスはバガザウイルス(BAGV)の特徴を示した(アフリカのBAGV DakAr B209株とのヌクレオチド相同性94.80%)。疾患急性期の脳炎患者から採取した血清は、15%(8/53)がBAGV中和抗体陽性を示した。これは、インドで分離されたBAGVの初の報告である。抗BAGV中和抗体の存在は、人間集団がBAGVに暴露されていたことを示唆する。

報告企業の意見	今後の対応
1996年のインドのケララ州における脳炎アウトブレイク時に患者がバガザウイルスに感染していたことが判明したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(5)

Journal of General Virology (2009), 90, 2644–2649 DOI 10.1099/vir.0.012336-0

Short Communication

Genetic characterization of Bagaza virus (BAGV) isolated in India and evidence of anti-BAGV antibodies in sera collected from encephalitis patients

Vijay P. Bondre,[1] Gajanan N. Sapkal,[1] Prasanna N. Yergolkar,[2] Pradip V. Fulmali,[1] Vasudha Sankararaman,[1] Vijay M. Ayachit,[1] Akhilesh C. Mishra[1] and Milind M. Gore[1]

[1]National Institute of Virology, Pashan, Pune 411 021, India

[2]National Institute of Virology, Bangalore Field Unit, Bangalore, India

Correspondence
Vijay P. Bondre
vpbondre@yahoo.com *or*
bondrevp@icmr.org

During investigations into the outbreak of encephalitis in 1996 in the Kerala state in India, an arbovirus was isolated from a *Culex tritaeniorhynchus* mosquito pool. It was characterized as a Japanese encephalitis and West Nile virus cross-reactive arbovirus by complement fixation test. A plaque reduction–neutralization test was performed using hyperimmune sera raised against the plaque-purified arbovirus isolate. The sera did not show reactivity with Japanese encephalitis virus and were weakly reactive with West Nile virus. Complete open reading frame sequence analysis characterized the arbovirus as Bagaza virus (BAGV), with 94.80 % nucleotide identity with African BAGV strain DakAr B209. Sera collected from the encephalitic patients during the acute phase of illness showed 15 % (8/53) positivity for anti-BAGV neutralizing antibodies. This is the first report of the isolation of BAGV from India. The presence of anti-BAGV neutralizing antibodies suggests that the human population has been exposed to BAGV.

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An outbreak of Japanese encephalitis (JE) was reported from the Allapuzza, Thiruvanthapuram and Kottayam districts of Kerala state, India, during 1996. Only 33 % (50/150) of the sera collected from hospitalized cases were confirmed as JE by immunoglobulin M (IgM) ELISA. Other clinical specimens were not available for further investigations. Entomological investigations during the outbreak were carried out and 184 mosquito pools collected from the affected area were processed for isolation in 2-day-old Swiss mice by the intra-cranial route (Rodrigues *et al.*, 1980; George *et al.*, 1984). One pool from *Culex tritaeniorhynchus* showed sickness in inoculated mice. Brains from sick mice were harvested and suspended in 10 % bovalbumin phosphate saline. The suspensions were stored at −70 °C and designated as the arbovirus isolate (96363). The isolate showed cross-reactivity with anti-JE virus (JEV) and anti-West Nile virus (WNV) immune sera in a complement fixation (CF) test (Pavri & Ghosh, 1969; Rodrigues *et al.*, 1980; Damle *et al.*, 1998). The isolate did not react with immune sera raised against other circulating arboviruses, including Chandipura (*Rhabdoviridae*), Sindbis (*Togaviridae*), Chikungunya (*Togaviridae*), Kyasanur forest disease (*Flaviviridae*), Batai (*Bunyaviridae*) and Dengue (*Flaviviridae*) viruses (Paul *et al.*, 1970; Rodrigues *et al.*, 1980; George *et al.*, 1984).

In this study, we present the genetic characterization of the arbovirus isolate and serological analysis of available sera collected from encephalitis patients during 1996. The Institutional Animal Ethical Committee approved this work and ethical guidelines were strictly followed according to their recommendations. The arbovirus isolate was plaque-purified to rule out the possibility of isolation of both JEV and WNV from the mosquito pool. The mouse brain stock of the arbovirus isolate was passaged twice in porcine stable kidney (PS) cells to amplify the virus. A single plaque was selected from the first PS cell passage and then subjected to two sequential rounds of plaque purification (total of three plaque-to-plaque transfers), followed by amplification in PS cells. The cell culture supernatant from PS cells was clarified by centrifugation at 3220 *g* for 10 min at 4 °C, supplemented with 20 % fetal bovine serum (FBS) and the aliquots were stored at −80 °C and designated as the arbovirus stocks. Generation of the arbovirus virus-specific polyclonal hyperimmune sera,

The GenBank/EMBL/DDBJ accession number of the Indian Bagaza virus isolate sequenced in this paper is EU684972.

A supplementary figure showing the phylogenetic analysis of BAGV based on nucleocapsid, membrane, non-structural (NS) 1, NS2, NS3, NS4 and NS5 gene sequences is available with the online version of this paper.

plaque reduction neutralization test (PRNT) and genetic characterization studies were performed using the PS-amplified arbovirus stocks. Since the CF test characterized the isolate as a JEV and WNV cross-reactive arbovirus, PRNTs were performed to determine the antigenic relationship among these viruses. An *in vitro* neutralization test was carried out using PS-adapted JEV (strain 733913), WNV (strain 804994) and the arbovirus isolate (strain 96363), as described previously (Bondre *et al.*, 2007). The threefold-diluted hyperimmune sera were mixed with 100 p.f.u. of each virus and the infectivity was determined in PS cells. The serum dilution showing 80 % plaque reduction (ND_{80}) was considered as a neutralizing end point. As shown in Table 1, the highest neutralizing activity was observed with homologous sera. In heterologous neutralization between the arbovirus isolate and WNV, both viruses showed cross-reactivity with each other, although this was weaker than the homologous neutralization. The JEV-specific hyperimmune sera did not neutralize the arbovirus isolate, even at a dilution of 1 : 5.

As the CF test characterized the 96363 isolate as a JEV- and WNV-reactive arbovirus and the heterologous neutralization showed that it had weak reactivity with WNV, we genetically characterized the isolate. A 1050 nt fragment from the NS5 region of the sample was amplified by RT-PCR using flavivirus-specific universal primers that amplify the partial NS5 fragment from a number of flaviviruses (Kuno, 1998). The genomic RNA of plaque-purified arbovirus grown in PS cells was isolated using QIAamp viral RNA kit (Qiagen) according to the manufacturer's protocol. The RT-PCR amplification was carried out as described by Kuno *et al.* (1998) and the amplified product was sequenced as described previously (Bondre *et al.*, 2007). BLAST analysis showed 99.90 % nucleotide identity (PNI) with African Bagaza virus (BAGV) strain DakAr B209, followed by 95 PNI with Israel turkey meningoencephalitis virus (ITMV). RT-PCR amplification and complete genome sequencing of BAGV-India was achieved by using overlapping primers designed by aligning available flavivirus sequences from GenBank with CLUSTAL_X 1.83 software (Thompson *et al.*, 1997). RT-PCR amplification of overlapping genomic fragments was carried out as described previously (Bondre *et al.*, 2007). PCR products were column-purified (QIAquick PCR purification kit; Qiagen) and both strands were sequenced by using a Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and an automated Sequencer (ABI Prism 310 Genetic Analyzer). A 10281 nt genomic sequence of BAGV-India (GenBank accession no. EU684972) coding a 3426 aa complete open reading frame (ORF) was obtained. Multiple alignments of nucleotide sequences were carried out by using CLUSTAL_X 1.83. The phylogenetic analysis of the complete genome sequence of BAGV-India was assessed by using MEGA (Tamura *et al.*, 2007). For analysis in MEGA, Jukes–Cantor and nucleotide maximum composite likelihood models were utilized, employing the neighbour-joining algorithm. The topologies generated in the neighbour-joining algorithm were confirmed by using the maximum-likelihood method, as implemented in the software Treefinder 2008, with the gamma-distributed rate variation with four rate categories (HKY+γ4) model of nucleotide substitution (Jobb *et al.*, 2004). The reliability of different phylogenetic groupings was evaluated by using the bootstrap test (1000 bootstrap replications). The genetic distance between different viruses was obtained by using the *P*-distance model in MEGA. Phylogenetic trees were constructed by using the complete genomic sequence of the Indian BAGV isolate (this study) and complete genomic sequences (from GenBank) of representative strains from different genomic groups in the *Flaviviridae*. Similarly, phylogenetic analysis of genomic fragments encoding different proteins – nucleocapsid, pre-membrane and membrane, envelope and non-structural (NS) proteins 1–5 – was carried out to understand the relationship between African and Indian BAGV isolates and other flaviviruses.

Comparative analysis of both the Indian and African (AY632545) BAGV complete ORF coding nucleotide sequences showed 94.8 (PNI). The difference of 515 nt (5.2 %) resulted in 77 aa (2.24 %) differences throughout the ORF of Indian and African (DakAr B209) BAGV isolates (Kuno & Chang, 2007). A difference of 20 aa was documented in the structural protein coding region (14 nt in the nucleocapsid with 2 aa differences, 40 nt in the membrane with 13 aa and 73 nt in the envelope with 5 aa), while a difference of 57 aa was documented in the NS protein coding region (71 nt in the NS1 region with 8 aa differences, 50 nt in the NS2 region with 7 aa, 95 nt in the NS3 region with 9 aa, 48 nt in the NS4 region with 19 aa and 119 nt in the NS5 region with 14 aa). Additionally, compared with BAGV-DakAr B209, one deletion (at nt 7424) and four additions (nt 7438–7439, 7444 and 7463) were documented in the NS4B region of BAGV-India.

Phylogenetic analysis using the complete sequence of the Indian BAGV ORF showed that this sequence had a close genetic relationship with the African BAGV-DakAr B209 strain and clustered together with the *Culex* mosquito-transmitted clade on the phylogram (Fig. 1). Similar tree topologies were obtained with both models (Jukes–Cantor and maximum composite likelihood) that were used to

Table 1. Homologous and heterologous cross-neutralization test using hyperimmune sera against JEV, WNV and arbovirus (BAGV) isolates

Serum giving 80 % plaque reduction was considered to be at the neutralizing end point. ND_{80} values are given.

Virus strain	Hyperimmune sera against:		
	JEV (733913)	WNV (804994)	BAGV (96363)
JEV	501	5	<5
WNV	<5	239	31
BAGV	<5	21	67

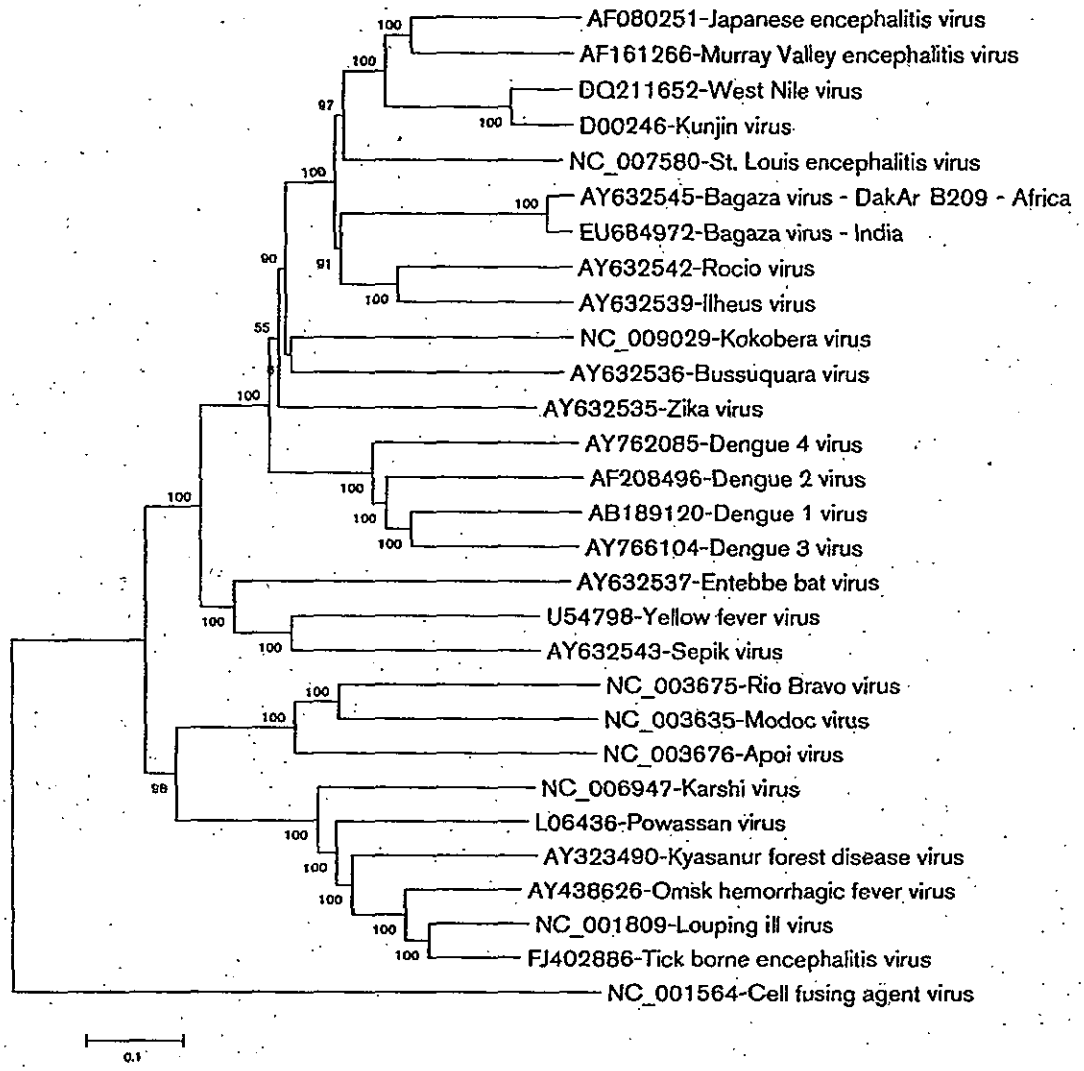

Fig. 1. Phylogenetic analysis of the BAGV complete ORF sequence using the nucleotide maximum composite likelihood model of the neighbour-joining algorithm. Cell fusing agent virus was used as an outgroup in phylogenetic analysis. GenBank accession numbers are given on the figure. Numbers at the nodes indicate bootstrap support for each node. Bar, nt substitutions per site.

construct the complete ORF sequence based on the phylogenetic tree obtained by using the neighbour-joining algorithm. The phylogenetic analysis of individual gene sequences coding for nucleocapsid, membrane, NS1, NS2, NS3 and NS4 showed similar tree topologies, which were comparable with complete genome sequence-based analysis (Supplementary Fig. S1, available in JGV Online). The PNI using nucleocapsid and membrane coding gene sequences of Indian and African BAGV isolates was 96.00 ± 1.25 and 92.30 ± 1.20, respectively. Analysis of the NS proteins NS1, NS2, NS3 and NS4 of both the BAGV isolates showed 94.40 ± 0.70, 95.10 ± 0.60, 95.20 ± 0.50 and 95.80 ± 0.60 PNI, respectively. A number of previous phylogenetic studies on flaviviruses mostly attempted to use envelope coding sequences. We also determined the genetic relationship of BAGV-India using the additional envelope sequences of representative members from different *Flaviviridae* groups. In envelope sequence-based analysis, BAGV-India grouped together with the African DakAr B209 strain (95.90 ± 0.80 PNI) along with other members of the Ntaya virus group of the *Flaviviridae* (Fig. 2). Envelope sequence analysis of the African BAGV strain (AF372407; Gaunt *et al.*, 2001) showed that it had a closer relationship (99.00 ± 0.40 PNI) with DakAr B209 strain than BAGV-India (94.80 ± 1.70 PNI). Among other members of the Ntaya virus group, ITMV showed a close relationship (93.40–95.50 PNI) with all three BAGV strains, followed by a more distant relationship with Ntaya virus (76.00–77.00 PNI) and Tembusu virus (74.00–75.00 PNI). As partial NS5 sequences from additional

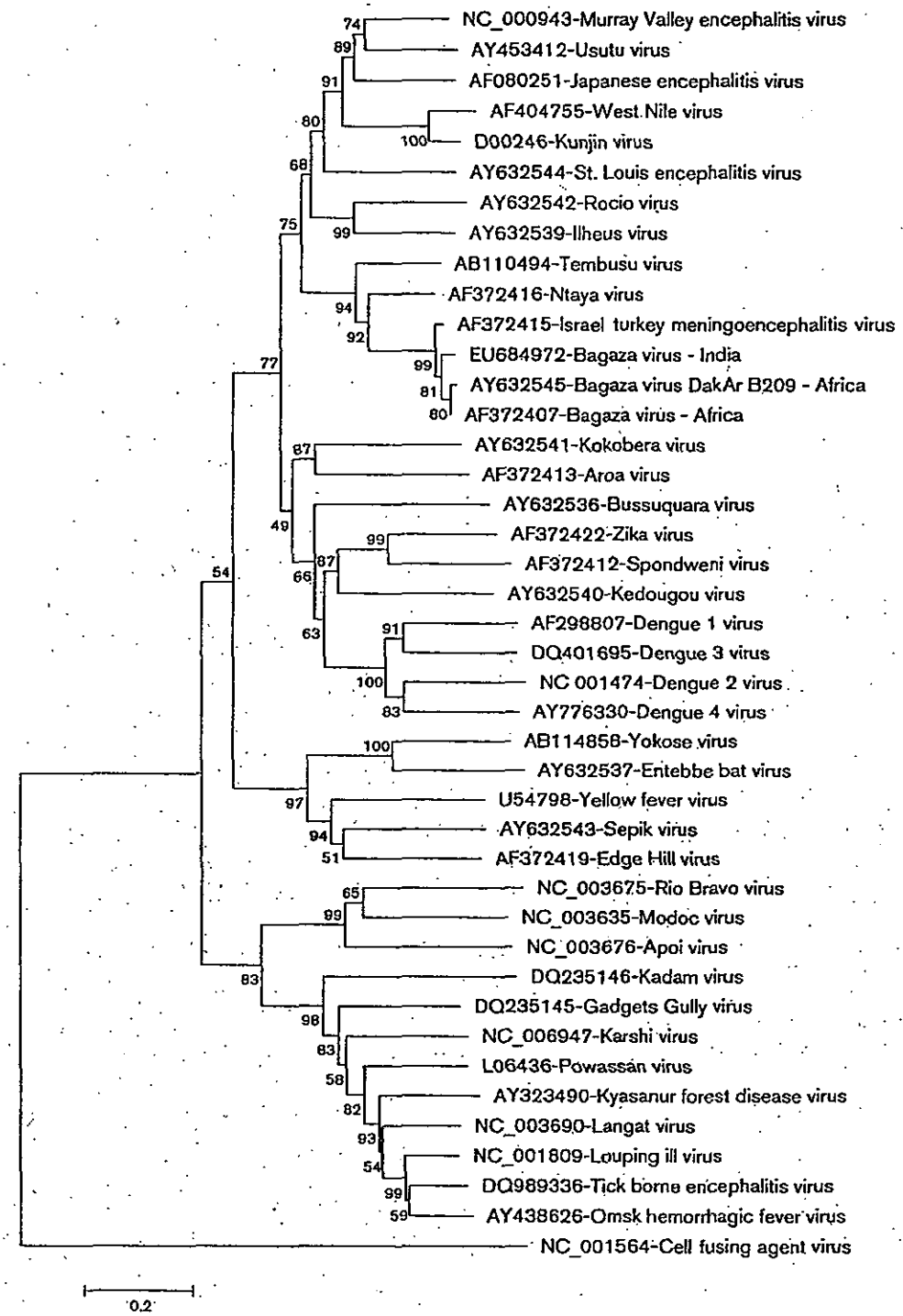

Fig. 2. Phylogenetic analysis of BAGV based on partial envelope sequences. The tree was constructed by using MEGA, by the neighbour-joining with nucleotide maximum composite likelihood model. Bootstrap confidence level (1000 replicates) and a confidence probability value based on the standard error test were calculated using MEGA and are indicated at the nodes. Partial envelope sequences of additional viruses (where complete genome sequences were not available) were used in the phylogenetic analysis. Cell fusing agent virus was used as an outgroup in phylogenetic analysis. GenBank accession numbers are given on the figure. Numbers at the nodes indicate bootstrap support for each node. Bar, nt substitutions per site.

members of the *Flaviviridae* were available in GenBank, we performed separate analysis to determine the genetic relationship of BAGV-India with these viruses (data not shown). With NS5 analysis, both the BAGV sequences grouped together, with 99.90±0.10 PNI, in the Ntaya virus group. However, in NS5 sequence analysis, the nucleotide identities of BAGV and other members of the Ntaya virus group were comparable with envelope sequence analysis. BAGV DakAr B209 and Indian strains showed 95.20–95.30 PNI with ITMV, 76.50–76.60 PNI with Ntaya virus and 75.10–75.30 PNI with Tembusu virus.

We documented one nucleotide insertion and four nucleotide deletions in the complete ORF sequence of Indian and African BAGV strains. The envelope sequence analysis of an additional BAGV strain from Africa indicates a closer genetic relationship with BAGV DakAr B209 than the Indian BAGV strain. These data indicate independent circulation of both the African and Indian isolates in different geographical areas. Although the time and mode of introduction of BAGV in India is unknown, we hypothesize that it may represent a genetic variant of the BAGV strain which originated in the African continent and was dispersed and established in areas with similar climatic conditions and favouring vector multiplication. Dispersal of the flaviviruses from the Old World to the New World and the co-existence of related viruses sharing antigenic, host and vector similarities have been supported by molecular phylogenetic analyses (Sabin, 1959; Gaunt *et al.*, 2001; Chevalier *et al.*, 2004; Mackenzie *et al.*, 2004; Petersen & Marfin, 2005; Gould *et al.*, 2006). However, to determine the precise genetic relationship, geographical origin and epidemiology, full genome sequence data of more strains will be helpful.

We isolated BAGV from a mosquito pool collected during a JE outbreak and studied its genetic relationship with other *Flaviviridae*. Since it was characterized as a JEV and WNV cross-reactive arbovirus (CF test), we determined the antigenic relationship with JEV and WNV by PRNT. Although the heterologous neutralization differentiated these as three distinct arboviruses, we documented weak cross-reactivity between WNV and BAGV (Table 1). The genetic relatedness of BAGV and WNV in several genomic regions might be the reason for antigenic cross-reactivity between these viruses (Kuno & Chang, 2007). We determined the previous exposure of hospitalized encephalitis patients with BAGV by analysing the sera stored at −80 °C for anti-BAGV neutralizing antibodies. The neutralization assay was performed with PS cell-adapted BAGV pools, as described previously (Bondre, *et al.*, 2007; Sapkal *et al.*, 2007). Only 15% (8/53) of available sera showed reactivity with BAGV, while 24.14% (14/53) were reactive with JEV (733913). Both the anti-JEV and anti-BAGV neutralizing antibody titres (ND_{80}) were in the range of 50–1250. All of the BAGV reactive sera were negative for JEV by IgM ELISA.

Recently, BAGV has been identified as one of the emerging and re-emerging human pathogens that causes febrile illness in humans (Woolhouse *et al.*, 2006). It belongs to the Ntaya group of *Flaviviridae* and has been isolated in the Central African Republic, Cameroon and Senegal, where it circulates between ornithophagic mosquitoes and birds (Digoutte, 1978; Traore-Lamizana *et al.*, 1994; Diallo *et al.*, 2005). It is genetically related to ITMV, which is a serious avian pathogen in the Middle East and southern Africa (Digoutte, 1978; Kuno *et al.*, 1998). The phylogenetic studies using envelope and NS5 sequences clearly suggest that there is a close genetic relationship between ITMV and BAGV. Other members of the Ntaya virus group are genetically distinct from BAGV and ITMV. Our preliminary findings on sera collected during the acute phase of illness from hospitalized patients indicates the presence of anti-BAGV neutralizing antibodies. This suggests that BAGV might be circulating in the area between ornithophagic mosquitoes and birds and incidentally the human population might be exposed to it. These observations need to be strengthened by investigating additional human clinical specimens from the region. However, our preliminary observations need to be confirmed by systematic study of the human population from the Allapuzza, Thiruvanthapuram and Kottayam districts of Kerala to understand the association of BAGV with human infections.

In conclusion, this study indicates the necessity of serious efforts to investigate the likely involvement of BAGV in sporadic human infections and outbreaks in other vertebrates occurring in the region. This can be achieved by developing BAGV-specific serological and molecular diagnostics for testing of human clinical specimens collected from the region. Additional studies addressing the potential of various mosquito species as vectors and birds as amplifying hosts, and sero-surveillance in domestic animals and the human population will add to our understanding of the epidemiology of arboviral diseases.

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No. 22

医薬品 研究報告 調査報告書

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				2009. 10. 7	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		Planitzer CB, Modrof J, Yu MY, Kreil TR. Emerg Infect Dis. 2009 Oct. Available from http://www.cdc.gov/EID/content/15/10/1668.htm	米国

研究報告の概要

○血液および血漿供血者の血漿中のウエストナイルウイルス感染(アメリカ合衆国)

2003～2008年に供給された米国の血漿由来静注用免疫グロブリン製剤(IGIV)中のウエストナイルウイルス(WNV)中和抗体価と最近の感染との関連を検討した。抗体価は供血者のWNV既感染率と密接に相関し、2008年ロットから既感染率は1%と推定された。

血漿由来IGIV製剤と、NATによる感染確定後の供血者由来血漿検体の中和抗体価を、古典的マイクロ中和試験により測定した。供血血液のWNVスクリーニング結果から1999年から2008年の各年における平均WNV感染数を算出した。米国疾病対策センターに報告された神経侵襲性症例数から推定し、その年の累積感染率を求めた。

IGIVのWNV中和抗体価は2003年から急速に増加し始めた。WNVスクリーニング結果から、2003年までに米国の人口の0.5%がWNVに感染したと推定された。米国の人口における既感染者の推定数は、IGIVの抗体価と平行して増加していた。2008年に出荷されたロットでは、中和抗体価は2.8～69.8、平均±SEMは21±1(n=256)であった。NATでWNV感染が確定した人から得られた血漿ではさらに抗体価が高く、検査した30名で平均±SEMは208±40となった。また、これらの結果から、米国の人口の1%が既にWNVに感染したと推定された。

米国の血漿由来IGIV製剤中の中和抗体価は上昇しており、特にWNV既感染供血者の抗体価が高いことから、WNVの予防や治療を目的としたIGIV製剤製造の可能性が示唆される。

報告企業の意見	今後の対応
2003～2008年に供給された米国の血漿由来静注用免疫グロブリン製剤中のウエストナイルウイルス(WNV)中和抗体価と最近の感染との関連を検討したところ、抗体価は供血者のWNV既感染率と密接に相関し、2008年ロットから既感染率は1%と推定されたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき緊急対応の準備を進めているほか、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(7)

West Nile Virus Infection in Plasma of Blood and Plasma Donors, United States

Christina B. Planitzer, Jens Modrof, Mei-ying W. Yu, and Thomas R. Kreil

This study investigated the association of ongoing West Nile virus (WNV) infections with neutralizing antibody titers in US plasma-derived intravenous immune globulin released during 2003–2008. Titers correlated closely with the prevalence of past WNV infection in blood donors, with 2008 lots indicating a prevalence of 1%.

West Nile virus (WNV) is a flavivirus endemic to the United States; typically, hundreds of clinical cases of infection occur each year. The observed number of clinical WNV infections as collated by ArboNET (www.cdc.gov) and the incidence of asymptomatic WNV infections as shown by nucleic acid testing (NAT) of the US blood supply (*1*) indicate that ≈3 million WNV infections occurred in humans during 1999–2008.

Because the immune system elicits WNV neutralizing antibodies in response to WNV infection, detectable levels of WNV neutralizing antibodies in the blood of persons with previous WNV infection is expected. Consequently, lots of immune globulin-intravenous (human) (IGIV) manufactured from plasma collected in the United States contain WNV neutralizing antibodies (*2*). Those IGIV lots, each prepared from several thousand plasma donations to ensure a broad spectrum of antibodies, can be used as an epidemiologic tool that enables the surveillance of thousands of persons in a community through analysis of comparatively few samples. In this study, we demonstrated the increasing trend of WNV-neutralizing antibody titers in lots of IGIV.

Comparing these titers with those of persons with confirmed past WNV infection provides an independent measure of the percentage of the US population previously infected with WNV. Several WNV vaccine trials are ongoing or imminent, so information about the prevalence of past WNV infection in the United States is valuable for planning the demonstration of vaccine efficacy. Low incidence and lack of highly WNV-endemic areas in the United States preclude classic vaccine field trials because of study size requirements and cost-logistics difficulties.

Author affiliations: Baxter Bioscience, Vienna, Austria (C.B. Planitzer, J. Modrof, T.R. Kreil); and US Food and Drug Administration, Bethesda, Maryland, USA (M.-y.W. Yu).

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The Study

The WNV neutralization titers of several US plasma–derived IGIV products (Gammagard Liquid/KIOVIG; Gammagard S/D/ Polygam S/D; Iveegam EN [Baxter Healthcare Corporation, Westlake Village, CA, USA]) and plasma samples obtained from US blood donors after a NAT-confirmed WNV infection were determined by an infectivity assay as earlier described (*2*), adapted to a classical microneutralization format (*3*). WNV neutralization titers (i.e., the reciprocal dilution of a 1:2 series resulting in 50% neutralization [NT_{50}; detection limits <0.8 for undiluted IGIVs and <7.7 for 1:10 prediluted serum]) are reported as the mean ± SEM. An unpaired *t* test was used to evaluate whether titer differences between 2 groups were statistically significant.

Using an extrapolation derived from screening the US blood supply for WNV (*1*), we calculated the average annual number of WNV infections in the United States for 1999–2008. The total number of neuroinvasive cases reported for those years to the US Centers for Disease Control and Prevention (CDC) through ArboNET was multiplied by 256 (i.e., the factor between all WNV infections and neuroinvasive cases). The cumulative infection rate for each year during 1999–2008 was then calculated by dividing the infections occurring up to a specific year by the US population for that year (determined by US Census Bureau estimates [www.census.gov/popest/states/NST-ann-est.html]).

Although WNV was first introduced into the United States in 1999, only in 2003 did the mean WNV neutralization titers of IGIV lots released to the market start to increase markedly (Figure 1). According to extrapolations from the WNV screening of the US blood supply (*1*), by 2003, an estimated 0.5% of the US population had been infected with WNV, although most infections were asymptomatic.

A delay of ≈1 year occurs between the collection of plasma and the release of IGIV lots to the market; thus, the WNV-positive IGIV lots in 2003 reflect the larger number of WNV infections occurring in 2002. Using the same extrapolations from the US blood supply (*1*), we found that the ≈0.1% annual increments in the proportion of the US population with past WNV infection follow a straight line ($r^2 = 0.9996$), generally paralleled by the mean WNV neutralization titers of IGIV lots. During 2005–2008, when large numbers of lots of a single IGIV product (Gammagard Liquid) could be analyzed, the WNV neutralization titer increased by 3.6 per year ($r^2 = 0.9793$).

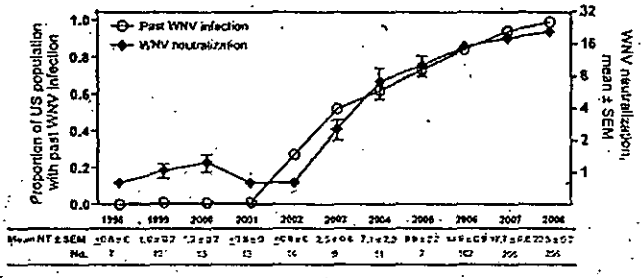

Figure 1. West Nile virus (WNV) neutralization titers of US plasma-derived immune globulin intravenous (human) (IGIV) lots by year of production and estimated percentage of the US population with past WNV infection by year. WNV neutralization titers were determined either for retention or lot release samples of 3 IGIV products produced during 1998–2005 or for a considerable proportion of Gammagard Liquid/KIOVIG lots produced during 2006–2008. Results are shown as mean ± SEM (limit of detection <0.8) by year of product release. For 5% of IGIV samples, titers were multiplied by 2 for comparison with the 10% IGIV samples at equivalent immunoglobulin concentrations. The percentage of the US donor population with past WNV infection was calculated from the number of neuroinvasive cases reported per year and the estimated ratio of neuroinvasive cases to total cases of WNV infection.

US plasma-derived IGIV lots released during 2008 showed variable WNV neutralization titers ranging from 2.8 to 69.8; mean ± SEM titer was 21 ± 1 (n = 256) (Figure 2). Compared with titers shown to be protective in an animal model of WNV infection (equivalent to >21 by the current assay) (*2*), ≈40% of the 2008 IGIV lots had higher titers.

Plasma obtained from persons with NAT-confirmed WNV infection had even higher titers; mean ± SEM titer was 208 ± 40 for 30 persons available for testing. When results were corrected for the immunoglobulin (Ig) G concentration in plasma (≈1%), compared with the 10% IGIV preparations, the mean neutralization titer of the plasma samples was ≈100× higher than that of the IGIV lots tested (2,080 vs. 21).

Conclusions

The most comprehensive collation of information about the incidence of WNV infection in the United States is available from ArboNET. When that information is combined with information obtained from the nationwide screening of the blood supply for WNV RNA by NAT (*1,4,5*), the current prevalence of past WNV in the US population is estimated to be ≈1%.

Busch et al. has noted that large-scale, community-based serologic surveys are hardly feasible because of their expense and because WNV ELISA assays are possibly biased by cross-reactions with other flaviviruses (*1*). Nevertheless, 7 seroepidemiologic studies have been performed (*6–12*). Cumulatively, 5,503 persons were tested for WNV infection by ELISA, and the results have shown highly divergent seroprevalence rates ranging between 1.9% (*6*) and 14.0% (*10*).

The use of IGIV lots, each representing the serostatus of several thousand donors in 1 sample, makes seroepidemiology practical (*13*) because it allows a large donor population to be surveyed by analyzing comparably few samples. The use of a more complex yet functional virus neutralization assay minimizes concerns about cross-reactivity with flaviviruses of other serocomplexes (e.g., dengue virus) that occasionally circulate in the US population. Also, epidemiologic considerations render interference by St. Louis encephalitis virus, a flavivirus within the same serocomplex, highly unlikely (*2*). The specificity of the neutralization assay was confirmed by testing IGIV lots manufactured from European-derived plasma against tick-borne encephalitis virus, a flavivirus closely related to WNV and circulating in Europe. Although these lots contained high neutralization titers against tick-borne encephalitis virus, only 1 of 20 had a detectable neutralization titer of 5 against WNV (unpub. data).

In this study, we determined that the mean titer of samples obtained during 2003–2008 from persons with a confirmed diagnosis of WNV infection was 100× higher than the mean titers of IGIV lots produced in 2008. This determination provides an independent experimental measure of the frequency of past WNV infection in the general US population, as reflected by the plasma/blood donor community, and the results correlate well with results of previously published theoretical extrapolations (*1*), which estimated that ≈1% of the population has already been infected with WNV.

The increasing levels of WNV neutralizing antibodies in IGIV lots from US plasma and the particularly high

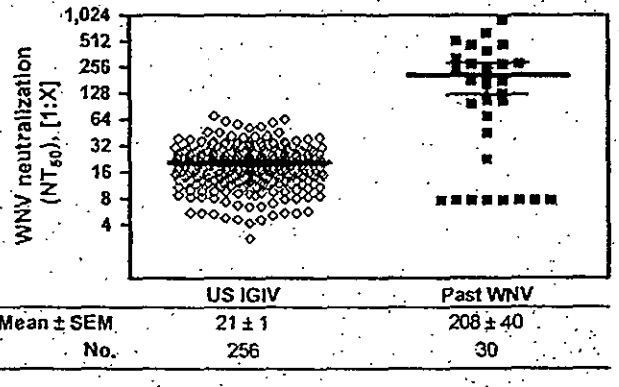

Figure 2. West Nile virus (WNV) neutralization by US plasma-derived immune globulin intravenous (human) (IGIV) released in 2008 and plasma from donors with past WNV infection (past WNV), confirmed by nucleic acid testing. WNV neutralization titers are shown as the mean ± SEM (limit of detection <0.8 for undiluted IGIVs and <7.7 for prediluted sera). NT_{50}, 50% neutralization titer.

titers in donors who have had a WNV infection suggest the possibility of preparing IGIV products with sufficiently high titers to be useful for WNV prophylaxis or treatment. Several ongoing or imminent WNV vaccine clinical trials stress the practical value of an independent confirmation of extrapolations that estimate the percentage of the US population with past WNV infection. Knowing the percentage of preexisting WNV seroprevalence as well as estimates of the mostly asymptomatic incidence rates (*14*) can be of vital importance in designing vaccine trials.

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Ms Planitzer is writing her PhD thesis on virus antibodies in immune globulins at the Global Pathogen Safety Group of Baxter BioScience in Vienna, Austria, in collaboration with the Medical University of Vienna, Austria. Her research focuses on determining functional antiviral properties of immunoglobulin preparations.

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Address for correspondence: Thomas R. Kreil, Baxter BioScience, Benatzkygasse 2-6, A-1221 Vienna, Austria; email: thomas_kreil@baxter.com

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販売名（企業名）	ハプトグロビン静注2000単位「ベネシス」　(ベネシス)					

研究報告の概要

このガイダンスは、輸血によるウエストナイルウイルス(WNV)伝播の可能性を最小化するために、血液採取施設が講ずる措置についての推奨である。示された全血及び血液成分のドネーションのための推奨内容は以下の通りである。、

A. 検査、ユニットの管理及びドナー管理

1. 輸注を意図して採取された全血および血液成分のドナーサンプルについて、WNVのスクリーニングを認可されたNATで1年を通じて行うことを推奨する。

2. 血液採取・取扱施設がミニプールNAT(MP-NAT)を用いてスクリーニングを行っているのならば、その施設は、陰性であったミニプールを構成している各試験サンプルのもとのユニット全てを、それらがWNV以外の点について出荷可とすることが適切であるのならば、出荷することができる。FDAは、血液採取・取扱施設がNATで陽性を示したミニプールを、それを構成する各検体に戻って個別NAT(ID-NAT)を用いて検査し、そのミニプールが陽性となる原因となったユニット(単数または複数)を同定することを推奨する。

B. MP-NATからID-NATへの切替え

1. 当該施設が採取を行う地域内で「WNVの活動性が高い」ということを定義する判断基準を設定し、バリデートすること。

2. 当該施設が採取を行う地域で「WNVの活動性が高い」際にMP-NATからID-NATへとスイッチするため、およびその地域での「WNVの活動性が高い」状態が収まったときにMP-NATへと戻すための閾値を定めること。

3. MP-NATからID-NATへのスイッチは可能な限り早期に行うべきだが、定めた閾値に達してから48時間以内に行うこと。

4. このような決定のプロセスについてSOP(標準作業手順書)を制定し、それに従うこと。

C. 検査実施報告書

1. 血液採取・取扱施設が認可を得ている施設であって、かつ、血液製剤の感染症検査を行うことがすでにFDAによって承認された施設では、認可を得ているWNV NAT検査を製造者の使用説明書に従って当該施設で用いることができ、その場合には21 CFR 601.12(d)に従って、検査法の変更について、その施設のFDAへの年次報告中に記載してFDAに知らせなければならない。

2. 血液採取・取扱施設が認可を得ている施設であってWNVのNAT検査を行うために新たな契約ラボを利用する場合であって、かつ、そのラボがすでに血液製剤の感染症検査を行っている場合には、その血液採取・取扱施設はその変更についてFDAに報告しなければならず、またそのことは21 CFR 601.12(c)(1)および(5)に従って"Supplement-Changes Being Effected"の申請を行うことによって報告しても良い。

D. 輸血を目的とした全血及び血液成分の表示

21 CFR 606.122(h)は、輸注を意図した血液製剤用の使用案内書("Circular of Information"としても知られている)には、安全でかつ有効な使用のために必要であれば、実施した検査名と結果を全て含めることを求めている。この21 CFR 606.122(h)に準拠するために、WNVのNATとして認可を受けた検査を実施するに際しては、認可を受けた血液採取・取扱施設、認可を受けていない施設のどちらでも、そのような使用案内書を改訂して、WNVについてのNAT検査が陰性であったとの結果を含めるようにしなければならない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意

(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

報告企業の意見	今後の対応	
ウエストナイルウイルスの伝播リスクを低減するための核酸検査（NAT）の使用に関する業界ガイダンスである。 FDA は、2005 年 6 月の業界向けガイダンス改訂版において、「FDA は全ての血漿分画製剤について現在行われているウイルス低減工程を再調査した。現在行われている方法は、WNV と分類上関連しているフラビウイルスを不活化することがバリデートされている。」と評価し、CPMP もまたポジションステートメントにおいて、血漿分画製剤の製造工程で WNV は不活化・除去されると評価している。万一、原料血漿に WNV が混入しても、BVD をモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。	

ハプトグロビン

Guidance for Industry

Use of Nucleic Acid Tests to Reduce the Risk of Transmission of West Nile Virus from Donors of Whole Blood and Blood Components Intended for Transfusion

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2009

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Contains Nonbinding Recommendations

Guidance for Industry

Use of Nucleic Acid Tests to Reduce the Risk of Transmission of West Nile Virus from Donors of Whole Blood and Blood Components Intended for Transfusion

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, FDA, are issuing this guidance to provide you[1] with recommendations for testing donations of Whole Blood and blood components for West Nile Virus (WNV) using an FDA-licensed donor screening assay[2]. We believe that the use of a licensed nucleic acid test (NAT) will reduce the risk of transmission of WNV, and therefore recommend that you use a licensed NAT to screen donors of Whole Blood and blood components intended for transfusion for infection with WNV.

The recommendations in section III of this guidance apply to all donations of Whole Blood (as defined in Title 21 Code of Federal Regulations (CFR) 640.1) and blood components for transfusion[3].

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

WNV first appeared in the United States in 1999, and has become endemic with high viral activity during the warm months of the year. WNV is a mosquito-borne agent that is maintained

[1] This guidance is intended for establishments that collect Whole Blood and blood components intended for transfusion.

[2] This guidance finalizes the recommendations for donations of Whole Blood and blood components in the draft guidance titled, *Guidance for Industry: Use of Nucleic Acid Tests to Reduce the Risk of Transmission of West Nile Virus from Donors of Whole Blood and Blood Components Intended for Transfusion and Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)*, dated April 2008 (April 28, 2008, 73 FR 22958).

[3] This guidance does not apply to Source Plasma or plasma derivatives.

in nature primarily between birds and mosquitoes but can also infect other animals, including humans. The potential for WNV transmission by blood transfusion during the acute phase of infection, when infected individuals are viremic and asymptomatic, was first recognized in 2002 (Ref. 1). At that time, test kit manufacturers and blood organizations, with input from the Public Health Service (National Institutes of Health, FDA, and Centers for Disease Control and Prevention (CDC)), actively pursued development of NAT systems for WNV. Retrospective studies have subsequently confirmed human-to-human transmission of WNV by blood transfusion and by organ transplantation (Refs. 2, 3).

Nationwide clinical studies to evaluate a NAT for the detection of WNV were initiated in 2003, under FDA's Investigational New Drug Application (IND) regulations (21 CFR Part 312). Such large-scale studies were necessary to help ensure blood safety and to determine the efficacy of investigational assays to prevent the transmission of WNV through blood transfusion, because at that time there was no FDA-licensed screening assay available to detect WNV infection.

Since 2005, FDA has approved biologics license applications for two NAT assays for detecting WNV ribonucleic acid (RNA) using plasma specimens from human donors of blood. The assays are intended for use in testing individual donor samples and in testing pools of human plasma comprised of equal aliquots of not more than either 6 or 16 individual donations (minipools) of whole blood and blood components, depending on the manufacturer.

As explained below in section III, if the result of a licensed minipool NAT (MP-NAT) is reactive, and subsequent testing of the individual donation(s) (ID-NAT) comprising the tested minipool is reactive, then FDA would recommend treating the reactive unit(s) as though they are infectious.

Evaluation of additional testing performed on specimens that were reactive on screening by ID-NAT has shown that a repeat ID-NAT on index donation specimens (i.e., the same or an independent specimen from the index donation, which is the donation for which the test result was reactive), using either the same screening assay or an equally sensitive alternate NAT, together with a test result for antibody to WNV, has a positive predictive value of 98% (Ref. 4).

Data show that up to 10% of donors who have a reactive ID-NAT that fails to be reactive on repeat testing by ID-NAT actually are infected, based on the presence of antibodies to WNV either in the index donation (ca. 8%) or on a follow-up test (ca. 2%) (Ref. 4). Therefore, additional testing that would include repeat testing by ID-NAT along with testing for antibody to WNV may be of value in donor counseling.

A. Whole Blood and Blood Components

In 2002, there were 23 confirmed cases of WNV transmission by blood or blood components (Ref. 3). Only six transmissions of WNV by transfusion were documented in 2003 (Ref. 5) following nationwide implementation of screening for WNV by MP-NAT under an IND in July 2003. Retrospective studies using ID-NAT to test MP-NAT non-reactive specimens collected during that season identified additional reactive donations and indicated that up to 25% of viremic units were not detected by MP-NAT, presumably due to low viral load (Ref. 6). Results

of these studies show that for detecting WNV, ID-NAT has greater sensitivity than MP-NAT.

As a result, ID-NAT may identify reactive donations not detected by MP-NAT. However, limitations in reagent availability, and personnel and logistical issues related to blood donor screening may not allow full implementation of ID-NAT. During the development and implementation of the ID-NAT test under IND, MP-NAT of plasma samples (pools of 6 or 16 samples), rather than ID-NAT, was the only feasible format for performing the test. In addition, testing using the MP-NAT format was similar to the assay platforms being used for human immunodeficiency virus type 1 (HIV-1) NAT and hepatitis C virus (HCV) NAT at that time. As reagent availability increases, technology advances, and personnel and logistical issues related to blood donor screening diminish, year-round ID-NAT testing of all donations of blood and blood components, using a licensed NAT, may become feasible and practical.

Although year-round ID-NAT testing of all blood and blood components may not be currently feasible, we believe that using ID-NAT instead of MP-NAT on a limited basis during periods of high WNV activity to maximize the benefit to the public health is more practicable. Statistical analyses were performed on the data from the retrospective studies described above to establish criteria for defining high WNV activity in a particular geographic region (Ref. 7). These criteria were used as a "trigger" for ID-NAT implementation and for reversion to MP-NAT testing when the high WNV activity in that region subsided. Since 2004, ID-NAT screening replaced MP-NAT screening in those geographic regions of high WNV activity during epidemic periods (Refs. 7, 8) when a threshold was reached. The threshold was usually based on the number of MP-NAT-reactive screening test results obtained during a one-week interval or on a cumulative rate for ID-NAT reactive screening test results in a particular region (Ref. 4).

After selective implementation of ID-NAT during epidemic seasons, there were three additional transmissions of WNV by transfusion between 2004 and 2006: one in 2004 and two in 2006. The WNV transmission in 2004 resulted from a donation of red blood cells which tested non-reactive in a MP-NAT assay, but which was subsequently found to be reactive in an ID-NAT test. Plasma from the donation retrospectively tested reactive by ID-NAT. However, ID-NAT had not yet been implemented (Ref. 9). The two WNV transmissions in 2006 resulted from a non-reactive MP-NAT donation from which red blood cells and fresh frozen plasma were transfused to two immunosuppressed recipients (Ref. 10). Investigation of the 2006 cases showed that: 1) there were no established methods of communication linking WNV MP-NAT data from multiple collecting and testing facilities serving overlapping or adjacent geographic areas; and 2) if efficient communication mechanisms had been in place, the corresponding collection area would have reached the threshold for switching to ID-NAT screening, and the WNV-contaminated components would likely have been detected and removed from the blood supply (Ref. 4).

At this time, there is insufficient data to support recommendation of uniform threshold criteria for switching from MP-NAT screening to ID-NAT screening. Pending development of suitable uniform threshold criteria, we consider it appropriate for each blood establishment to define its own threshold criteria for switching from MP-NAT to ID-NAT screening and for reverting to MP-NAT screening. Each blood establishment should follow an established standard operating procedure (SOP) for this decision process. Voluntary industry practice of switching from MP-

NAT to ID-NAT screening during seasonal activity has been useful in increasing the effectiveness of the WNV screening process.

III. RECOMMENDATIONS FOR DONATIONS OF WHOLE BLOOD AND BLOOD COMPONENTS

Testing donations of Whole Blood and blood components for WNV using NAT involves the use of defined pooling and testing systems. We recognize that licensed testing technology in a semi-automated or fully automated format is not universally available, and that if you are currently performing NAT for WNV under an IND you would need time to fully implement a licensed system with all approved components, including the supporting software cleared as a device. If you are therefore using some, but not all, of the licensed or cleared components, you should continue your existing IND and report the use of the licensed assay or the related cleared components as an amendment to your existing IND. When you implement all licensed or cleared components of the test system, you may withdraw the IND in accordance with the procedures provided in 21 CFR 312.38.

A. Testing, Unit Management, and Donor Management

1. We recommend that you screen year-round for WNV using a licensed NAT on donor samples of Whole Blood and blood components intended for transfusion. In general, you may use either MP-NAT or ID-NAT for screening (see Figure 1 and Table 1), except that we recommend that you use ID-NAT screening during high WNV activity in your region (using a previously defined geographic area). See section B.

2. If you perform screening using MP-NAT, you may release all units whose test samples comprise a non-reactive minipool, if those units are otherwise suitable for release.

   We recommend that you resolve a NAT-reactive minipool using ID-NAT to test each specimen in the minipool in order to identify the unit(s) that led to the reactivity of the minipool. Based on the ID-NAT results, we recommend the following:

   a. You may release all ID-NAT non-reactive units if they are otherwise suitable for release.

   b. If one or more individual donation(s) is (are) reactive, we recommend that you discard the unit(s), defer the donor(s) for a period of 120 days and retrieve and quarantine in-date products from prior collections dating back 120 days prior to the donation that is ID-NAT-reactive. We recommend that you notify the donor of his or her deferral and counsel the donor. Further testing on the index donation using the same ID-NAT or an alternate NAT with sensitivity equal to or greater than that of the screening assay, in addition to testing the

specimen using a cleared test for antibodies to WNV may be of value in donor counseling.

Note: In the event that the NAT screening assay does not discriminate between WNV and other Flaviviruses that belong to the Japanese Encephalitis (JE) serogroup (namely, Saint Louis Encephalitis virus, Japanese Encephalitis virus, Murray Valley Encephalitis virus and Kunjin virus), the donor should be counseled that he or she tested positive for a JE serogroup virus, most likely WNV. Alternatively, the use of a NAT assay that discriminates WNV from other members of the JE serogroup may be of value in donor counseling.

Note: Antibodies to viruses of the JE serogroup may cross-react on the test for antibodies to WNV (Refs. 11, 12). Therefore, reactivity in a WNV antibody test may not be conclusive for WNV infection.

3. If you perform screening using ID-NAT, we recommend that you follow the steps in 2.a. and 2.b. for testing, unit management, and donor management.

B. Switching from MP-NAT to ID-NAT

We recommend that you:

1. Establish and validate criteria that define high WNV activity in your geographic area of collection.
2. Define a threshold for switching from MP-NAT to ID-NAT screening during high WNV activity in your geographic area of collection, and for reverting to MP-NAT screening when the high WNV activity in your geographic area has subsided.
3. Switch from MP-NAT to ID-NAT screening as soon as feasible, but within 48 hours of reaching that threshold.
4. Establish and follow an SOP for this decision process.

NOTE: To define the geographic area for which the threshold criteria would apply, you may consider using the donor's residential zip code or county, or other well-specified region of comparable size that includes the donor's residence. Although exposure to WNV may occur in any location, it is reasonable to assume that exposure most likely occurred while the donor was near his or her residence, because mosquito activity is highest at dawn and dusk, times when many donors are at home. Mechanisms for switching to ID-NAT screening that utilize defined geographic areas based on residential zip codes, counties, or other comparable well-specified regions provide a standardized method for collecting data on the number of NAT-reactive donations and the number of donations tested.

Consideration of other epidemiological data may be useful in defining a threshold for switching from MP-NAT to ID-NAT screening, if such data are available.

Examples include the number of clinical cases, the number of positive birds or mosquito pools reported in a particular geographic area, and prior ID-NAT implementation history.

You should switch from MP-NAT to ID-NAT screening when the WNV case threshold has been met or exceeded in your defined geographic area. Blood establishments that share geographic collection areas should consider a communication plan so that data from overlapping and adjacent collection areas may be shared and used to assess WNV activity in a defined geographic area. You may use this data to determine whether your defined threshold for switching to ID-NAT screening has been met.

C. Reporting Test Implementation

1. If you are a licensed blood establishment and are already FDA-approved to perform infectious disease testing of blood products, you may use at your facility a licensed WNV NAT according to the manufacturer's product insert, and you must notify us in your annual report of the testing change in accordance with 21 CFR 601.12(d). Also, if you have already filed a supplement to your Biologics License Application to use a contract laboratory to perform infectious disease testing of blood products, and the contract laboratory will now perform a NAT for WNV, you must report this change in your annual report, in accordance with 21 CFR 601.12(d).

2. If you are a licensed blood establishment and you use a new contract laboratory to perform a NAT for WNV and the laboratory already performs infectious disease testing for blood products, then you must report this change to FDA, and may do so through submission of a "Supplement – Changes Being Effected" in accordance with 21 CFR 601.12(c)(1) and (5), also known as changes being effected immediately (CBE). If your contract laboratory previously has not performed infectious disease testing for blood products, then you must submit this change in a prior approval supplement (PAS) in accordance with 21 CFR 601.12(b).

D. Labeling of Whole Blood and Blood Components Intended for Transfusion

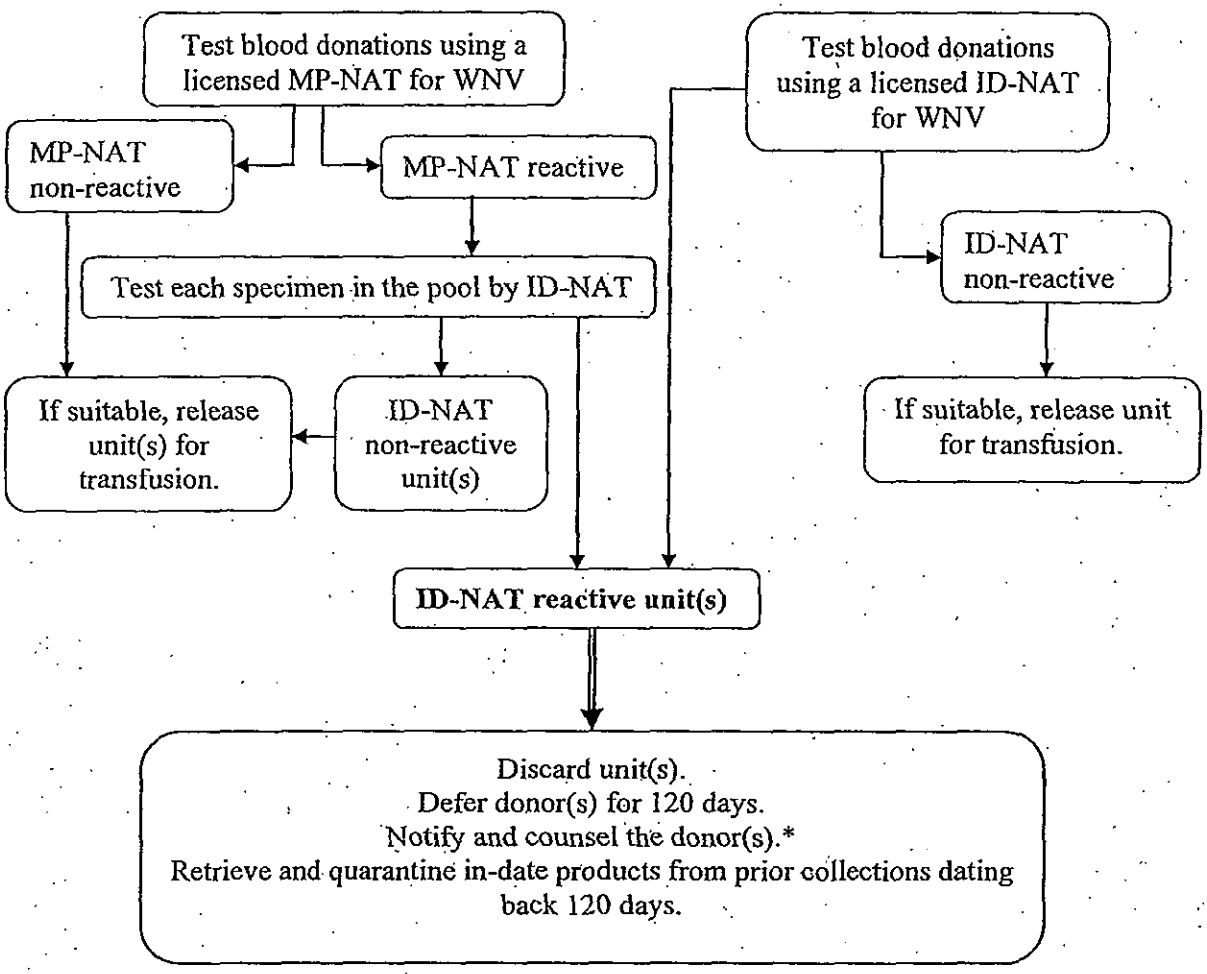
Title 21 CFR 606.122(h) requires that an instruction circular, also known as the "Circular of Information," for blood products intended for transfusion include the names and results of all tests performed when necessary for safe and effective use. To comply with 21 CFR 606.122(h), upon implementation of a licensed NAT for WNV, both licensed and unlicensed blood establishments must revise such instruction circular to include the non-reactive results of a NAT for WNV. If you are a licensed blood establishment, you may submit this labeling as a CBE (21 CFR 601.12(c)(1) and (5)), provided the revision is identical to the following statement:

"A Licensed Nucleic Acid Test (NAT) for West Nile Virus (WNV) RNA has

been performed and found to be non-reactive."

If you are a licensed blood establishment and you wish to use a different statement, then you must submit the labeling change as a PAS (21 CFR 601.12(b)). If you are an unlicensed blood establishment, you must revise the instruction circular under 21 CFR 606.122(h), but you are not required to submit the revision as a supplement.

Figure 1. Recommendations on Testing, Unit Management, and Donor Management for Whole Blood and Blood Components



* Additional testing on the index donation using the same ID-NAT assay or an alternate NAT of comparable sensitivity in addition to a cleared test for antibodies to WNV may be of value in donor counseling.

Note: In the event that the NAT screening assay does not discriminate between WNV and other Flaviviruses that belong to the Japanese Encephalitis (JE) serogroup (namely, Saint Louis Encephalitis virus, Japanese Encephalitis virus, Murray Valley Encephalitis virus and Kunjin virus), the donor should be counseled that he or she tested positive for a JE serogroup virus, most likely WNV. Alternatively, the use of a NAT assay that discriminates WNV from other members of the JE serogroup may be of value in donor counseling.

Note: Antibodies to viruses of the JE serogroup may cross-react on the test for antibodies to WNV (Refs. 11, 12). Therefore, reactivity in a WNV antibody test may not be conclusive for WNV infection.

Table 1. Recommendations on Testing, Unit Management, and Donor Management for Whole Blood and Blood Components

MP- NAT	ID-NAT	Actions
Reactive	Reactive unit(s)	Discard the unit(s).
		Defer the donor(s) for 120 days.
		Notify and counsel the donor(s).*
		Retrieve and quarantine in-date products from prior collections dating back 120 days.
	Non-Reactive unit(s)	If suitable, release units for transfusion.
Non-Reactive	Not needed	If suitable, release units for transfusion.

* Additional testing on the index donation using the same ID-NAT assay or an alternate NAT of comparable sensitivity in addition to a cleared test for antibodies to WNV may be of value in donor counseling.

Note: In the event that the NAT screening assay does not discriminate between WNV and other Flaviviruses that belong to the Japanese Encephalitis (JE) serogroup (namely, Saint Louis Encephalitis virus, Japanese Encephalitis virus, Murray Valley Encephalitis virus and Kunjin virus), the donor should be counseled that he or she tested positive for a JE serogroup virus, most likely WNV. Alternatively, the use of a NAT assay that discriminates WNV from other members of the JE serogroup may be of value in donor counseling.

Note: Antibodies to viruses of the JE serogroup may cross-react on the test for antibodies to WNV (Refs. 11, 12). Therefore, reactivity in a WNV antibody test may not be conclusive for WNV infection.

IV. IMPLEMENTATION

We recommend that you implement the recommendations in this guidance as soon as feasible, but not later than six months after the guidance issue date.

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番号：2

別紙様式第２－１

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 平成22年1月5日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ヨウ化人血清アルブミン（^{131}I）		研究報告の公表状況	Google News Dec 18, 2009 （追加情報：The New York Times December 19,2009）	公表国 米国	
販売名（企業名）	放射性ヨウ化人血清アルブミン注射液（富士フイルムＲＩファーマ株式会社）					
研究報告の概要	要約：米国においてアメーバ性髄膜脳炎の初めてのヒトからヒトへの伝播が報告された： 米国にて、臓器ドナーから腎移植を受けたレシピエント２例がアメーバ性髄膜脳炎に感染し、患者は重篤な状態にあることが保健当局により報告された。これは、おそらく初めてのヒトからヒトへのアメーバ性髄膜脳炎の伝播であると思われる。レシピエント２例は重症であるが、同患者からの別の臓器の移植を受けたその他の患者は症状を呈していない。後に検査により、ドナーと腎移植を受けたレシピエント２例から髄膜脳炎の原因とされる「Balamuthia mandrillaris」というアメーバが検出され、感染が確認された。					使用上の注意記載状況・その他参考事項等 特になし
報告企業の意見			今後の対応			
初めての人から人への感染報告であり、新たな感染経路かつ重大な感染症に関するものであるため、感染症定期報告の対象と判断する。			本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連しないことから、現時点で当該生物由来製品に関し、措置等を行う必要はないと判断する。			

MedDRA/J Version(12.1)

8

CDC: Rare infection passed on by Miss. organ donor

By HOLBROOK MOHR (AP) – Dec 18, 2009

JACKSON, Miss. — An extremely rare infection has been passed from an organ donor to at least one recipient in what is thought to be the first human-to-human transfer of the amoeba, medical officials said Friday.

Four people in three states received organs from a patient who died at the University of Mississippi Medical Center in November after suffering from neurological problems, said Dave Daigle, a spokesman for the Centers for Disease Controls and Prevention.

Organs are routinely tested for HIV, hepatitis and other more common infections, but occasionally rare ones slip through.

"We test for the known harmful diseases, but there's not a test for every single pathogen out there," said Dr. Kenneth Kokko, medical director of kidney transplants at UMMC.

Two of the recipients are critically ill, but the others haven't shown symptoms, Daigle said. The CDC confirmed the presence of the organism, known as Balamuthia mandrillaris, in one of the recipients.

Dr. Shirley Schlessinger, a UMMC doctor and medical director of the Mississippi Organ Recovery Agency, would not say which states had patients receiving the organs.

The public should not be concerned, both Schlessinger and Daigle said.

Balamuthia mandrillaris is a microscopic parasite found in soil that causes encephalitis in humans, horses, dogs, sheep and nonhuman primates. Scientists think people get infected by breathing it in, but it can also pass into the blood through a cut or break in the skin. It can be especially dangerous to people undergoing organ transplants, whose immune systems are purposely weakened so their bodies don't reject their new organs.

Human infections are very rare: Only about 150 cases have been reported worldwide since the disease was first identified in 1990. But it can be hard to diagnose because few laboratories test for it and many doctors don't know about it. Some cases are not identified until autopsy, according to the CDC.

"The thing we don't want to happen is for people to take this rare and extraordinary anomaly and think it speaks to a lack of safety," she said. "It's very rare so the likelihood that this will happen again (is small), I mean, it's rarer than rabies."

There are risks to transplants and doctors can't test for everything, but the potential benefits far outweigh the risks, she said.

AP Medical Writer Mike Stobbe in Atlanta contributed to this report.

On the Net:

- CDC details on Balamuthia mandrillaris: http://bit.ly/7swHMV
- University of Mississippi Medical Center: http://www.umc.edu/

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CDC: Rare infection passed on by Miss. organ donor
The Associated Press - Dec 18, 2009

The New York Times

December 19, 2009

2 Kidney Recipients Contract Brain Disease From Donor

By DENISE GRADY

Two transplant patients are critically ill with a rare brain infection that was transmitted to them by kidneys taken from a donor at the University of Mississippi Medical Center in Jackson, health officials reported on Friday.

The same infection probably killed the organ donor, but it was not diagnosed; his doctors thought he had an autoimmune disease. Two other patients also received heart and liver transplants from the donor, but neither has become ill. The transplants took place in November, in three states. A spokeswoman for the university declined to say where the recipients were, citing patient confidentiality.

Three weeks after their transplant surgeries, the kidney recipients became ill abruptly, within hours of each other, with seizures, a change in mental status and fever, said Dr. Eileen Farnon, an epidemiologist at the Centers for Disease Control and Prevention, which is investigating the cases. A doctor noted that both were transplant recipients, and immediately suspected that they might have contracted an illness from the donor.

Subsequent tests of tissue left from the deceased donor found the infection, which was also diagnosed in the patients. The patients are being treated with a mixture of antimicrobial drugs.

The infection is caused by an amoeba, Balamuthia mandrillaris, which lives in soil and water. Only about 70 cases have ever been identified in the United States. Nearly all have been fatal. The current cases are the first to have been found in transplant recipients. Although infections from transplants are uncommon, there have been cases in which recipients contracted West Nile virus, rabies and other infections.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 10. 14	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		水野泰孝, 氏家無限, 竹下望, 加藤康幸, 金川修造, 工藤宏一郎, 林昌宏, 高崎智彦. 第58回日本感染症学会東日本地方会学術集会・第56回日本化学療法学会東日本支部総会合同学会; 2009 Oct 30-31; 東京.	日本

研究報告の概要

○遷延する関節痛を主訴に来院したチクングニヤ熱の3例

チクングニヤ熱(Chikungunya fever;CHIKF)は発熱、関節炎、発疹を主症状とする熱性疾患であり、臨床症状や検査所見はデング熱に類似するが、遷延する関節症状が特徴的である。本年になり東南アジア地域を中心に再びCHIKF流行が拡大しており、2009年5月から6月にかけての2ヶ月間で、東南アジアから帰国後に遷延する関節痛を主訴に来院した3例を血清学的にCHIKFと診断したのでその概要を報告する。(症例1)52歳日本人男性。2009年3月26日から4月5日までインドネシア・スマトラ島へ蝶の採集目的で滞在。3月31日に39.5度の発熱、関節痛(両手足首、両膝)が出現。翌日には解熱したものの関節痛は持続したため、帰国後5月上旬に近医整形外科受診。関節リウマチ、痛風検査を実施されるも陰性であったため、精査目的で当センターを受診した。(症例2)30歳日本人男性。2009年4月16日より6月14日までインドネシア・ジャワ島へ舞台公演目的で滞在。5月13日に発熱、関節痛(右足首、左膝、右肩)、頭痛、発疹が出現。4日後に解熱したものの関節痛は持続したため6月22日に当センターを受診した。(症例3)39歳日本人女性。2009年4月4日より6月28日までマレーシア・クアラルンプール郊外に帯同家族として滞在。5月12日に39.5度の発熱、関節痛(両手足首)、発疹、歯肉炎が出現。現地の病院で膠原病スクリーニング等の精査を受け、異常所見は認められなかったものの関節痛が持続するため、6月30日に当センターを受診した。いずれの症例も来院時の検査でチクングニヤウイルスIgM抗体及び中和抗体陽性であり、血清学的にCHIKFと確定診断した。流行地から帰国した後、遷延する関節症状を訴える患者を診療する場合には、リウマチ性疾患との鑑別の上でもCHIKFの可能性を考慮に入れた正確な血清診断を行うべきである。

報告企業の意見	今後の対応
2009年5月から6月にかけて、東南アジアから日本へ帰国後に、遷延する関節痛を主訴に来院した3例を血清学的にチクングニヤ熱と診断したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

041

在日ラテンアメリカ人の慢性シャーガス病キャリアーと2次感染予防

慶應義塾大学医学部熱帯医学寄生虫学

○三浦 左千夫, 竹内 勤

我が国の在日ラテンアメリカ人は既に40万人に達する勢いで増加している。そのうちブラジルからの滞在者が80%を占めており、その8万人が既に定住永住権を取得している。こうした中で、南米特有の風土病シャーガス病患者も散見されるようになった。近年各地医療機関から依頼のあった心疾患患者41名についてシャーガス病病原体*Trypanosoma cruzi* (*T.cruzi*) 血清抗体検査を行った。その結果15名 (36.59%) が明らかに陽性と判定され、シャーガス病が示唆された。更に、抗体陽性者について血液を材料にしたPCRを行った結果4名に*T.cruzi*－DNA産物を検出した。病原体の血液内生残が強く示唆されたので、更に血液培養を試みた結果2名 (抗体陽性者の13.3%) から*T.cruzi*虫体を分離することが出来た。即ち慢性の病原体キャリアーが日本に現存することが明らかとなった。ECGでは不整脈、心エコーで拡張型心筋症を示した。ブラジル、ボリビアの生活歴がある者に関しては、我が国では臨床経験の少ないシャーガス病感染を検討すべきである。

一方、消化器系の症状を訴える患者の検査依頼は皆無であったが、心室拡張症で通院している同一患者は消化器症状 (飲み込み困難、排便困難) をも訴えているものの、検査を受けていない。

本疾患の特徴は感染者の70%は病型が定まらない慢性感染で、一見健常者とみえることである。本人、家族もその感染を認知するものは少ない。

媒介昆虫の存在しない日本国内で感染が起こるとすれば、それは輸血感染、臓器移植による2次感染であると思われる。肝要な点は、事前の抗体チェックでこのような2次感染が防げることである。ラテンアメリカ人の多くを抱える地方自治体は健康保健支援環境を整備し、シャーガス病の2次感染を阻止すべく啓蒙監視活動を強化すべきであり、全国的に行われている善意の献血現場で抗体スクリーニングを実施すべく、体制の整備を行う必要がある。

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遷延する関節痛を主訴に来院したチクングニヤ熱の3例

[1]国立国際医療センター戸山病院 国際疾病センター, [2]長崎大学熱帯医学研究所 臨床医学分野, [3]国立感染症研究所 ウイルス第一部

○水野 泰孝[1], 氏家 無限[1,2], 竹下 望[1], 加藤 康幸[1], 金川 修造[1], 工藤 宏一郎[1], 林 昌宏[3], 高崎 智彦[3]

チクングニヤ熱 (Chikungunya fever; CHIKF) は発熱、関節炎、発疹を主症状とする熱性疾患であり、臨床症状や検査所見はデング熱に類似するが、遷延する関節症状が特徴的である。本年になり東南アジア地域を中心に再びCHIKF流行が拡大しており、当センターにおいても2009年5月から6月にかけての2ヶ月間で、東南アジアから帰国後に遷延する関節痛を主訴に来院した3例を血清学的にCHIKFと診断したのでその概要を報告する。(症例1) 52歳日本人男性。2009年3月26日から4月5日までインドネシア・スマトラ島へ蝶の採集目的で滞在。3月31日に39.5度の発熱、関節痛 (両手足首、両膝) が出現。翌日には解熱したものの関節痛は持続したため、帰国後5月上旬に近医整形外科受診。関節リウマチ、痛風検査を実施されるも陰性であったため、精査目的で当センターを受診した。(症例2) 30歳日本人男性。2009年4月16日より6月14日までインドネシア・ジャワ島へ舞台公演目的で滞在。5月13日に発熱、関節痛 (右足首、左膝、右肩)、頭痛、発疹が出現。4日後に解熱したものの関節痛は持続したため6月22日に当センターを受診した。(症例3) 39歳日本人女性。2009年4月4日より6月28日までマレーシア・クアラルンプール郊外に帯同家族として滞在。5月12日に39.5度の発熱、関節痛 (両手足首)、発疹、歯肉炎が出現。現地の病院で膠原病スクリーニング等の精査を受け、異常所見は認められなかったものの関節痛が持続するため、6月30日に当センターを受診した。いずれの症例も来院時の検査でチクングニヤウイルスIgM抗体及び中和抗体陽性であり、血清学的にCHIKFと確定診断した。流行地から帰国した後、遷延する関節症状を訴える患者を診療する場合には、リウマチ性疾患との鑑別の上でもCHIKFの可能性を考慮に入れた正確な血清診断を行うべきである。

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 9. 16	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		ProMED 20090831.3065, 2009 Aug 31. 情報源:Thanhnien News.com, 2009 Aug 29.	ベトナム

研究報告の概要

○デング/デング出血熱 最新情報
[1]ベトナム
ベトナムの首都ハノイの保健当局は、年初～8月下旬のデング熱患者数が2500名近くに達したと報告した。これは、2008年の同時期の10倍以上となり、ここ数年で最悪の状態である。国立感染症・熱帯医学研究所の医師によると、患者の大半は市内のHoang Mai、Thanh Xuan、Dong Daなどの地区で発生しており、2008年に隣接の地区を合併して人口が増えたために患者が増加したという説を否定した。
南部のホーチミン市では、2009年の症例数は大きく増えてはいないものの、重症化・死亡する患者が多くなっている。市の保健当局によると、年初～現在までの症例数は7100例で2008年の同時期と比べて5%多く、死亡患者は現時点で7名となっている。同市の第一小児病院では治療を受けている80名以上の子供のうち、1/4は循環器障害、神経学的問題、出血などを発症するステージ3か4である。毎日20～25名の子供が入院しており、70%はホーチミン市の患者である。「症状が出て1～2日の間は、手足口病やH1N1インフルエンザと区別がつきにくい。H1N1では様々な症状が現れるため、デングへの警戒がおろそかになっているが、子供は死に至る危険性がある」と第一小児病院の医師は警告した。

報告企業の意見	今後の対応
ベトナムの首都ハノイで、デング熱患者が2008年の同時期の10倍以上に達し、南部のホーチミン市では重症化・死亡する患者が多くなっているとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(10)

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Archive Number 20090831.3065
Published Date 31-AUG-2009
Subject PRO/EDR> Dengue/DHF update 2009 (35)

DENGUE/DHF UPDATE 2009 (35)
****
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

In this update:
[1] Viet Nam
[2] Sri Lanka
[3] Myanmar (Rakhine)
[4] India (Gujarat)
[5] Pakistan
[6] Mauritius
[7] Dominican Republic

******
[1] Viet Nam
Date: Fri 29 Aug 2009
Source: Thanhnien News.com [edited]

Earlier this week, the Hanoi Health Department reported that nearly 2500 cases of the mosquito-borne illness had been recorded citywide since the beginning of the year [2009], 10 times more than over the same period last year [2008]. The department said the figures were the worst in years. "The number of dengue cases has gone up critically," said National Institute of Infectious and Tropical Diseases deputy director Nguyen Hong Ha, adding that the institute had admitted up to 45 dengue patients a day recently. "Around 100 patients are receiving treatment at the institute right now' we're on overload," he said. "Patients have to share beds, and we've even had to set up beds in the corridors. But that's still not enough; ... we're buying more beds."

According to the doctor, most of the patients had come from inner-city districts like Hoang Mai, Thanh Xuan, and Dong Da. He said that fact refuted the popular theory that the number of Hanoi dengue patients had gone up because the capital had absorbed parts of Hoa Binh and Vinh Phuc in 2008.

Although the number of dengue cases recorded in Ho Chi Minh City hasn't increased sharply this year [2009], more patients have reached critical condition, and there have been more deaths related to dengue, said Dr. Phan Van Nghiem from the city's health department. Over 7100 cases have been recorded citywide since the beginning of the year [2009], an increase of 5 percent compared with the same period last year [2008]. The city has already seen 7 deaths due to dengue, according to the department.

Doctor Le Bich Lien, head of the Dengue Fever Department at Children Hospital No. 1, said her facilities were treating around 80 kids for dengue, 1/4th of whom were in stages 3 and 4 with symptoms like circulatory failure, neurological problems and hemorrhaging. "Around 20-25 children are admitted to the hospital with dengue fever every day," said Dr. Lien. "HCMC kids account for 70 percent of our child patients."

Between 50 and 60 kids, mainly 3-10 years old, are receiving treatment

for dengue at HCMC Children Hospital No. 2, said Dr. Tran Thi Thuy, deputy head of the hospital's Infection Department. "Around 10 percent of them are in phase 4, the most critical phrase, and experiencing physical shock," Dr. Thuy said.

Thu Duc General Hospital has reported that some 20-30 dengue patients, mainly adults, were currently undergoing treatment there.

"During the 1st 1-2 days of infection, dengue in kids is difficult to distinguish from hand-foot-mouth disease or H1N1 flu," Lien said. "As H1N1 flu manifests itself in complicated ways, many people have let their guard down against dengue fever. But dengue can be fatal for kids," she warned.

[Byline: Thanh Tung-Lien Chau]

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Communicated by:
ProMED-mail Rapporteur Mary Marshall

A map of Viet Nam showing the provinces can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/vietnam_admin01.jpg>. An interactive HealthMap/ProMED-mail of Viet Nam can be accessed at <http://healthmap.org/r/008c>. - Mod.TY]

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[2] Sri Lanka
Date: Sat 29 Aug 2009
Source: Xinhua News Agency [edited]
<http://news.xinhuanet.com/english/2009-08/29/content_11961338.htm>

The number of dengue cases has risen to 24 629 while 245 people have died of the disease in Sri Lanka so far this year [2009], the Epidemiological Unit of the Health Ministry said on Friday [28 Aug 2009].

The Epidemiological Unit said in its latest statistics that of the 24 629 cases, the highest number of patients were reported from June [2009] totaling 7048. It is followed by July [2009] with 6858 cases being reported.

This represents a sharp increase, as only 4156 dengue cases and 85 deaths were reported for the whole year of 2008.

Health officials said the majority of these cases have been reported from the areas of Kandy, Kegalle, Colombo, Gampaha and Kurunegala.

The rapid rise in the level of the epidemic has forced the health authorities to carry out extensive public awareness campaigns to eradicate the mosquito-based epidemic.

Households have been warned to keep the environment free of mosquitoes. Those who allow the mosquitoes to breed by allowing stagnating water face prosecution, with a special hotline being made available for public information.

There has been a decline in the number of dengue fever cases in August [2009], with 2387 cases being recorded as of [28 Aug 2009], officials said.

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Communicated by:
PRO/MBDS <promed-mbds@promedmail.org>

[During 2004 to 2009, the dengue outbreak in 2009 is the largest in Sri Lanka. Based on the above newswire, there have been 24 629 cases and 245 deaths so far (January-August 2009). The case fatality rate (CFR) is 0.99 percent. The number of reported dengue cases has dramatically increased nearly 6-fold as compared to 2008 (4156 cases).

At present, the trend of the dengue outbreak in Sri Lanka is decreasing, as there were 7048 cases, 6858 cases and 2378 cases reported in June, July and August 2009, respectively. However, more dengue outbreaks are also possible in November to February, when the

northeast monsoon begins.

Dengue is transmitted by the main vector, the _Aedes aegytpi_ mosquito. There are 4 distinct (but closely related) viruses that cause dengue. According to WHO's Regional Office for Southeast Asia (WHO/SEARO) report (available at <http://www.searo.who.int/en/Section10/Section332_1100.htm>), Sri Lanka, Indonesia, Thailand and Timor-Leste are classified in category A upon the transmission potential of dengue. The common characteristics among those countries are dengue fever (DF)/dengue haemorrhagic fever (DHF) as a major public health problem, which is the leading cause of hospitalization and death among children, and there are cyclical epidemics in urban centers and spreading to rural areas with multiple virus serotypes circulating.

In 2004, the total of dengue cases reported was 15 408 with 88 deaths (CFR 0.57) in Sri Lanka. During the past 20 years, the outbreak in 2004 was most serious, although the CFR was lower than in the past. Cases were reported every month, the highest being in June-July 2004. Cases were reported from 25 districts. Of these, 72 percent of cases and 78 deaths were from 5 cities, namely Colombo, Kandy, Gampaha, Kalutara and Kurunegala. The CFRs range from 0.4 percent to 1.1 percent.

In 2006, the reported dengue cases and deaths due to dengue had increased 2-fold as compared to 2005. The case fatality was maintained below one percent. In 2007 till May, 1846 dengue cases and 9 deaths have been reported from Sri Lanka (see <http://www.searo.who.int/en/Section10/Section332/Section2277_11963.htm>).

A map of Sri Lanka can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/sri_lanka_pol01.jpg>. A HealthMap/ProMED-mail interactive map of Sri Lanka can be accessed at <http://healthmap.org/r/009M>. - Mod.SCM]

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[3] Myanmar (Rakhine)
Date: Mon 24 Aug 2009
Source: Mizzima News [edited]
<http://www.mizzima.com/news/inside-burma/2666-dengue-kills-three-afflicts-over

According to information from the Ministry of Health, at least 3 people have died and 329 have been infected with dengue fever this year [2009] in Sittwe and Kyaukphyu of Arakan [Rakhine] State in western Burma [Myanmar].

According to the ministry of health, 2 people in Sittwe, capital of Arakan [Rakhine] state, have died and another in Kyaukphyu town.

"Though dengue is not very dangerous, 2 people died in our town, scaring people. There are many dengue afflicted child patients in hospital, but I cannot tell the exact number. Besides, there are many more unreported cases in the villages. The villagers cannot afford treatment at the hospital. Only the affluent in the town can get admitted to the hospital. Dengue has infected not only children but adults as well. There are many people from different age groups being treated at our hospital. Most patients are children, and the fever lasts less than a week, after which the patient is out of danger," a doctor in Sittwe Hospital said.

But some patients need to be treated for over a week. "My daughter had dengue since the beginning of this month [August 2009] and was hospitalized as soon as she was infected. Now she has been discharged. Though her condition has improved, she has not yet fully recovered. She has been absent from school for over 2 weeks," [her mother] in Sittwe told Mizzima.

Teachers are worried about their students, as many are absent from schools. "There are many children who cannot come to school because of the flu. Their friends say they either have flu or dengue fever. Some could not come to school for a whole month [August 2009]. We are worried about their education given the long absence from classes," a class teacher in the State High School No. 2 in Sittwe told Mizzima.

Though the symptoms of this disease are coughing, sneezing, fever, and

body ache, in this type of influenza, similar symptoms are not found, and there are only sudden high fever plus headaches.

Rash, bleeding from the nose and gums, bloodstains in the urine and stool were found in these patients. Patients are known to become unconscious, have convulsions, perspire with high fever, vomit continuously, and suffer from shock.

Dengue fever cases were also reported in Pyi, Pa-an in Karen State and Htantalan town in Chin State.

The Health Ministry release said that about 30 people die of dengue fever in Rangoon [Yangon] annually.

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PRO/MBDS <promed-mbds@promedmail.org>

[The newswire above is the 4th report of dengue cases and deaths in Myanmar since mid June 2009. However, it is the 1st report from Rakhine state (formerly Arakan), one of 7 states of Myanmar situated along the western coast. According to the newswire, there have been 329 dengue fever cases with 3 fatalities (2 cases from Sittwe and another one from Kyaukphyu town) during 2009.

The previously reported dengue outbreak in Myanmar occurred in Myitkyina, capital of Kachin State (see prior PRO/MBDS posting Dengue Myanmar (03): RFI 20090728.2650). There are no current reports of morbidity and mortality statistics in the country with respect to dengue fever in 2009. However, as of 24 Jul 2009, there were 838 cases with 6 deaths of dengue during 2009 in Yangon, Myanmar (see prior PRO/MBDS posting Dengue - Myanmar (02): Yangon 20090726.2635).

In Myanmar, dengue fever (DF)/dengue haemorrhagic fever (DHF) is one of the leading causes of morbidity and mortality among children under the age of 10 years, with approximately 85 percent of cases occurring in this age group. An annual average of 7000-10 000 cases of DF/DHF are reported nationwide. However, in recent epidemic years (2001, 2005, and 2007), the number had risen to over 15 000 cases. In 2007, 62 percent of all reported cases were from Yangon Division (31 percent), Ayeyarwaddy Division (16 percent) and Mon State (15 percent) (1).

The 1st major epidemic of the disease syndrome in Myanmar occurred in the capital, Yangon in 1970. Since then, epidemics have continued to occur in a cyclic pattern, and the disease has spread from Yangon to most parts of the country. Between 1970 and 1995, there were 83 381 cases of DHF with 3243 deaths, a case fatality rate of 3.88 percent. During the 1st 5 years in which DHF was known to occur in the country, almost all the cases were confined to the Yangon division. By 1975, the disease syndrome had begun to spread and, in that year, 31 percent of the DHF cases occurred in Mandalay and only 29 percent in Yangon. However, Yangon still remains the most serious focus of DHF (2).

According to WHO's Regional Office for South-East Asia (WHO/SEARO) report available at <http://www.searo.who.int/EN/Section10/Section332/Section2277_11962.htm>, in 2005 the total dengue cases reported was 17 454 and 169 deaths in Myanmar, and the case fatality rate was maintained below one percent. The increase in case load and deaths compared to 2004 is almost 2 times. In 2006, the reported dengue cases and deaths were reduced as compared to 2005. The case fatality rate in 2006 was slightly above one percent. The seasonal trend shows July as the peak month, and cases start increasing from May to peak in July-August.

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2. Prasittisuk C, Andjaparidze AG, Kumar V. WHO South-East Asia Regional Office: Current Status of Dengue/Dengue Haemorrhagic Fever in WHO South-East Asia Region. Dengue Bulletin Volume 22, December 1998 (available at

<http://www.searo.who.int/en/Section10/Section332/Section520_2414.htm>).

For maps of Myanmar see
<http://www.worldatlas.com/webimage/countrys/asia/lgcolor/mmcolor.htm>
and
<http://www.lib.utexas.edu/maps/middle_east_and_asia/burma_pol_96.jpg>. For the interactive HealthMap/ProMED-mail map of Myanmar with links to other ProMED-mail reports in Myanmar and surrounding countries, see <http://healthmap.org/r/00IU>. - Mod.SCM]

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[4] India (Gujarat)
Date: Mon 31 Aug 2009
Source: Times of India [edited]
<http://timesofindia.indiatimes.com/NEWS/City/Rajkot/Dengue-outbreak-gets-sever

The dengue outbreak in the city is refusing to die down, with 15 cases reported in the city in the past 48 hours. With 3 fresh cases reported on Sunday [30 Aug 2009], the total number of patients being treated for the disease in the city has gone up to 55. One person has died of the disease till date. The patients were admitted from Sukhnathpara, Sardarnagar and Baharwadi areas. On Saturday [29 Aug 2009], there were 7 new cases reported.

"We are doing our best to tackle the situation in the city. The district collector PR Sompura has formed a special team to root out the virus from the city. Daily, 10 teams under this special team are conducting door-to-door surveys along with officials from Amreli municipality, to find out cases," a district health official said.

Apart from health officials, teams from the municipality are also conducting cleanliness drives throughout the city. "We are fumigating all streets of the city every evening to kill mosquitoes carrying the dengue virus and cleaning any water-logged areas. However, our job will get more challenging with the 2nd spell of rainfall that has begun since the past 72 hours," an official from Amreli Nagar Palika said.

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[Fumigating the streets will be of only temporary value. Eliminating the vector mosquito breeding sites in and around houses and other buildings will provide more effective control of the outbreak.

An interactive map of Gujarat, India showing the location of Amreli and vicinity can be accessed at
<http://www.maplandia.com/india/gujarat/amreli/amreli/>. A HealthMap/ProMED-mail interactive map of India can be accessed at <http://healthmap.org/promed/en?v=22.9,79.6,5>. - Mod.TY]

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[5] Pakistan
Date: Wed 26 Aug 2009
Source: The News [edited]
<http://www.thenews.com.pk/print1.asp?id=195030>

Out of 18 patients of a locality admitted to Holy Family Hospital Saturday [22 Aug 2009] evening, 5 were declared positive for dengue fever by the National Institute of Health (NIH), Islamabad. The confirmation of 5 cases as positive, the 1st in this season in this region of the country, has convinced a number of health experts in town to fear an outbreak of the infection.

"The confirmation of 5 cases proved the existence of _Aedes aegypti_, the female mosquito that causes dengue fever [transmits dengue viruses] in town. The deaths of 2 children on Friday night and Saturday morning [21 and 22 Aug 2009] due to fever in the area from where 18 patients have been taken might be attributed to dengue fever or DHF," said NIH.

A special team of the District Health Department headed by District Health Officer Dr. Khalid Randhawa has shifted some 16 children and 2 adults to the HFH after suspecting them cases of dengue fever on Saturday evening [29 Aug 2009] from a village not more than 25 km from here. The team was constituted after the Executive District Officer (Health) received reports of deaths of the 2 children. The deceased as well as all the suspects admitted at HFH have been living in a cluster of nearly a dozen families settled near the village Larr in Dhoke Jhando, located in union council Thatta Khalil of Taxila.

All the 5 cases confirmed so far for the infection range between 3 and 8 years of age. The HFH has sent blood samples of a total of 18 suspected patients of dengue fever to NIH for dengue serology, of which 5 have been confirmed positive, 9 negative, while results of 4 cases have not been finalised as yet.

Experts do believe that with the detection of 5 confirmed cases in the outskirts of twin cities of Islamabad and Rawalpindi, a rising threat of an outbreak of dengue and DHF seems to be lurking, as the disease has a tendency to occur in epidemics and outbreaks and spreads like wild fire.

Head of Pathology Department at Rawalpindi Medical College Professor Dr. Abbas Hayat has repeatedly expressed to "The News" that the spikes of dengue fever, if they occur repeatedly, might be more deadly and might result in severe complications, including hemorrhagic manifestations. Two months back, he said that the situation might be alarming after the monsoon, as the climate after monsoon is considered to be the most suitable for the breeding of the mosquito _Aedes aegypti_ that causes [transmits the viruses that cause] DF and DHF. DHF is a cause of disease and death primarily among children in tropical Asia.

Studies have revealed that people at a higher risk for dengue transmission are children, travellers and tourists, whereas adults residing in endemic areas are also susceptible to contracting the disease.

The District Health Department has already claimed that it has performed fogging and sprinkled insecticidal spray in and around Larr; however, experts believe that a continuous surveillance is needed at this time to avert a possible outbreak of dengue fever.

[Byline: Muhammad Qasim]

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Communicated by:
HealthMap Alerts via
ProMED-mail <promed@promedmail.org>

[A HealthMap/ProMED-mail interactive map of Pakistan can be accessed at <http://healthmap.org/promed/en?v=30,69.4,5>. - Mod.TY]

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[6] Mauritius
Date: Thu 17 Aug 2009
Source: Eurosurveillance [edited]
<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19314>

[The following article presents an interesting approach for mapping dengue outbreaks. - Mod.TY]

Abstract
During the month of June 2009, Mauritius experienced a short-lived outbreak of dengue fever localised in its capital city Port Louis. _Aedes albopictus_, a secondary vector of dengue viruses, was the probable vector. We introduce a method which combines Google Earth images, stochastic cellular automata and scale free network ideas to map this outbreak. The method could complement other techniques to forecast the evolution of potential localised mosquito-borne viral outbreaks in Mauritius and in at-risk locations elsewhere for public health planning purposes.

Reference;

Ramchurn SK, Moheeput K, Goorah SS. 2009. An analysis of a short-lived outbreak of dengue fever in Mauritius. Euro Surveill 14:19314. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19314>.

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Communicated by:
ProMED-mail <promed@promedmail.org>

[A HealthMap/ProMED-mail interactive map of Mauritius can be accessed at: <http://healthmap.org/promed/en?v=-20.3,57.9,5>. - Mod.TY]

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[7] Dominican Republic
Date: Fri 28 Aug 2009
Source: El Nuevo Diario [in Spanish, trans. Mod.TY, edited]
<http://elnuevodiario.com.do/app/article.aspx?id=165814>

A dozen people, including adults and children, are affected by dengue, with one of these in a serious state, reported the representative of the municipal district Canca La Reina, Licenciado Manuel Antonio Rojas. The district executive said that the dreaded dengue outbreak that hit the different communities of Canca la Reina is produced by a strong wave of mosquitoes left by the passage of recent rains that have fallen in the past weeks. He recalled that in 2003, 4 people died in this community, affected by dengue, which is the reason that a call was issued to the provincial Health Directorate so that urgent measures would be taken together with the municipal government to avoid a repetition of that history. Tony Rojas said that the municipal government has maintained operations to eradicate trash, mosquito breeding sites and wells where the mosquito that is the dengue vector breeds.

He pointed out that the outbreak has become present in various communities of Canca La Reina, but the main effects have occurred in the Manhattan sector, where there is an affected child in an extremely serious state. "We have called Public Health on other occasions to carry out work against the dengue vector mosquito, but they have not reciprocated," complained Representative Tony Rojas. He said that the situation is very serious because there are more than 12 people affected by dengue.

Representative Tony Rojas stated that the municipal government is coordinating an urgent operation to tackle the epidemic of mosquitoes, stressing that the health of the population of Canca La Raina is in danger.

[Byline: Arcadio B. Rojas]

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Communicated by:
HealthMap Alerts via
ProMED-mail <promed@promedmail.org>

[A HealthMap/ProMED-mail interactive map showing the Dominican Republic and its location in the Caribbean can be accessed at <http://healthmap.org/promed/en?v=18.9,-70.5,5>. - Mod.TY]

[see also:
Dengue/DHF update 2009 (34) 20090823.2977
Dengue/DHF update 2009 (33) 20090817.2908
Dengue/DHF update 2009 (32) 20090811.2864
Dengue/DHF update 2009 (31) 20090803.2723
Dengue/DHF update 2009 (29) 20090720.2574
Dengue/DHF update 2009 (28) 20090713.2501
Dengue/DHF update 2009 (27) 20090706.2425
Dengue/DHF update 2009 (26) 20090629.2353
Dengue/DHF update 2009 (25) 20090622.2286
Dengue/DHF update 2009 (24) 20090614.2211
Dengue/DHF update 2009 (23) 20090608.2121
Dengue/DHF update 2009 (22) 20090601.2040
Dengue/DHF update 2009 (21) 20090525.1952
Dengue/DHF update 2009 (20) 20090518.1868
Dengue/DHF update 2009 (19) 20090512.1774

Dengue/DHF update 2009 (18) 20090505.1677
Dengue/DHF update 2009 (17) 20090428.1595
Dengue/DHF update 2009 (16) 20090419.1485
Dengue/DHF update 2009 (14) 20090406.1341
Dengue/DHF update 2009 (13) 20090331.1227
Dengue/DHF update 2009 (12) 20090314.1049
Dengue/DHF update 2009 (08) 20090216.0650
Dengue/DHF update 2009 (06) 20090210.0603]
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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 9. 16	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		
			Aguilar PV, Camargo W, Vargas J, Guevara C, Roca Y, Felices V, Laguna-Torres VA, Tesh R, Ksiazek TG, Kochel TJ. Emerg Infect Dis. 2009 Sep;15(9):1526-8.	公表国 ボリビア
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			

研究報告の概要	使用上の注意記載状況・その他参考事項等
○ボリビア出血熱の再興(2007～2008年) ボリビア出血熱(BHF)は、1959年に、ボリビア東部でのアウトブレイク発生時に初めて報告された。しかし、病原体のマチュポウイルスが死亡患者の脾臓から分離されたのは、1963年であった。1976～1993年は症例が報告されなかったが、1994年にアウトブレイクが起こり、以降、散発症例が観察されていた。 2007年の2月、3月に、BHF疑い症例20例以上(死亡3例)がボリビア北東部ベニの保健当局(SEDES)に報告されていた。2008年2月には、疑い症例200例以上(死亡12例)がSEDESに報告された。 疑い例患者から採取した血清19検体について、間接免疫蛍光法とPCRで検査を行った。アレナウイルスの分離株が5株得られ、遺伝子配列の解析からマチュポウイルスであることが確認された。	解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
2007年の2月、3月に、ボリビア出血熱の疑い症例20例以上、2008年2月には200例以上が報告され、19例について検査したところ、5例でマチュポウイルスが確認されたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

Table. Characteristics *Mycobacterium bovis* BCG complication cases, Taiwan, 2005–2007*

Patient no.	Sex/age at diagnosis, y	Year reported	Specimen	Diagnosis and site of involvement
1	F/2	2005	Biopsy sample	BCG osteitis/osteomyelitis, right ankle
2	M/1	2005	Bacterial isolate	Subcutaneous abscess, left anterior chest wall
3	M/2	2005	Bacterial isolate	Severe combined immunodeficiency, disseminated BCGitis
4	M/9	2005	Bacterial isolate	Suppurative lymphadenitis
5	F/1	2005	Bacterial isolate	Injection-site abscess
6	M/1	2005	Biopsy sample	Suppurative lymphadenitis
7	M/2	2006	Bacterial isolate	BCG osteitis/osteomyelitis, right distal femoris
8	M/2	2006	Bacterial isolate	BCG osteitis/osteomyelitis
9	F/1	2006	Bacterial isolate	BCG osteitis/osteomyelitis, left distal femoris
10	F/1	2006	Bacterial isolate	BCG osteitis/osteomyelitis, left distal radius
11	F/2	2007	Bacterial isolate	BCG osteitis/osteomyelitis, right knee
12	M/1	2007	Bacterial isolate	Subcutaneous abscess, left wrist
13	M/2	2007	Biopsy sample	BCG osteitis/osteomyelitis, right ankle
14	F/1	2007	Bacterial isolate	Suppurative lymphadenitis
15	M/2	2007	Bacterial isolate	BCG osteitis/osteomyelitis, left proximal tibia

*BCGitis, disseminated BCG infection.

age. In particular, suspected childhood TB patients without an identifiable TB contact and with normal immune status were subjected to further investigations. Multidisciplinary management, including enhanced laboratory diagnosis of atypical bony lesions in infants and children, is recommended for any suspected TB infection. Once BCG-related infection is confirmed, medical treatment has to be consistent.

Acknowledgments

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Ruwen Jou, Wei-Lun Huang, and Wei-Ju Su

Author affiliation: Taiwan Centers for Disease Control, Taipei, Taiwan

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Address for correspondence: Ruwen Jou, Reference Laboratory of Mycobacteriology, Research and Diagnostic Center, Taiwan Centers for Disease Control, Department of Health, 161 Kun-Yang St, Nan-Kang, Taipei, 115, Taiwan, Republic of China; email: rwj@cdc.gov.tw

Reemergence of Bolivian Hemorrhagic Fever, 2007–2008

To the Editor: Bolivian hemorrhagic fever (BHF) was first described in 1959 during outbreaks affecting isolated human communities in eastern Bolivia. However, it was not until 1963 that the etiologic agent, Machupo virus, was isolated from the spleen of a patient who died from this disease (*1*). Although no cases were reported between 1976 and 1993, an outbreak occurred in 1994 and sporadic cases have been observed since then.

In February and March 2007, at least 20 suspected BHF cases (3 fatal) were reported to the El Servicio Departamental de Salud (SEDES) in Beni,

Bolivia. In February 2007, physicians at the Hospital Santa Maria Magdalena reported 3 male patients (23, 27, and 29 years of age), who worked at a ranch in Magdalena, Itenez Province (13°14′0″S, 64°12′0″W). The patients sought treatment for fever, gingivorrhagia, petechiae, nausea, hematemesis, melena and tremors; clinical laboratory examinations showed thrombocytopenia (<130,000 cells/mm^3), leukopenia (<3,900 cells/mm^3), and hematuria. Because physicians suspected BHF, patients received supportive therapy, including intravenous hydration, corticoids, antipyretic drugs, antimicrobial drugs, and blood transfusions from donors who had survived Machupo virus infection. Nonetheless, 2 of the patients died 3 and 4 days after admission.

In February 2008, at least 200 suspected new BHF cases (12 fatal) of BHF were reported to SEDES. A febrile hemorrhagic illness developed in a 19-year-old man from Huacaraje, Itenez Province (13°33′S, 63°45′W). On first examination at the Hospital Santa Maria Magdalena, the patient had fever, tremor, gingivorrhagia, petechiae, bruises, asthenia, and anorexia and was admitted with a presumptive diagnosis of BHF. Despite supportive treatment (including administration of plasma from a BHF survivor), his condition worsened; hematemesis, melena, hematochezia, hematuria, anuria, respiratory alkalosis, and metabolic acidosis developed in the patient, eventually resulting in death. A fifth case was detected in a 46-year-old man from San Ramon, Mamore Province (13°17′0″S, 64°43′0″W). A febrile hemorrhagic illness developed in the patient and he was admitted to the Hospital German Busch in Trinidad. The patient recently had been hired as a farm worker. When first seen by the attending physicians, he had fever, thrombocytopenia, leukopenia, petechias, tremors, gingivorrhagia, and dehydration, consistent with symptoms of BHF. The patient received hydration, corticoids, antipyretic therapy, and a plasma transfusion from a BHF survivor. The patient's condition improved and he was subsequently discharged from the hospital ≈10 days after admission.

Nineteen serum samples collected from suspected BHF patients, including the cases described above, were sent to Centro Nacional de Enfermedades Tropicales (Santa Cruz, Bolivia) and the US Naval Medical Research Center Detachment (Lima, Peru) for testing. Serum was injected into Vero and C6/36 cells; 10 days later, the cells were tested for flaviviruses, alphaviruses, and arenaviruses by indirect immunofluorescent assay and PCR. Five arenavirus isolates were obtained from the patients described in this report.

Viral RNA was extracted from the cell culture supernatant and the small (S) segment (≈3,200 bp) was amplified and sequenced. Phylogenetic analyses were conducted using the neighbor-joining and maximum likelihood program implemented in PAUP 4.0 software (Sinauer Associates, Inc., Sunderland, MA, USA). Sequence analyses confirmed the isolates as Machupo virus (Figure). Eight major Machupo phylogenetic lineages were described based on partial sequence of the nucleocapsid protein gene (*2*). We observed a similar tree topology based on the glycoprotein gene sequences (Figure). Two distinct lineages were distinguished among the isolates from the Itenez and Mamore provinces: V and VII and I and II, respectively. The recent isolates (2007–2008) from Magdalena and Huacaraje (Itenez Province) grouped within lineage V whereas the 2008 isolate from San

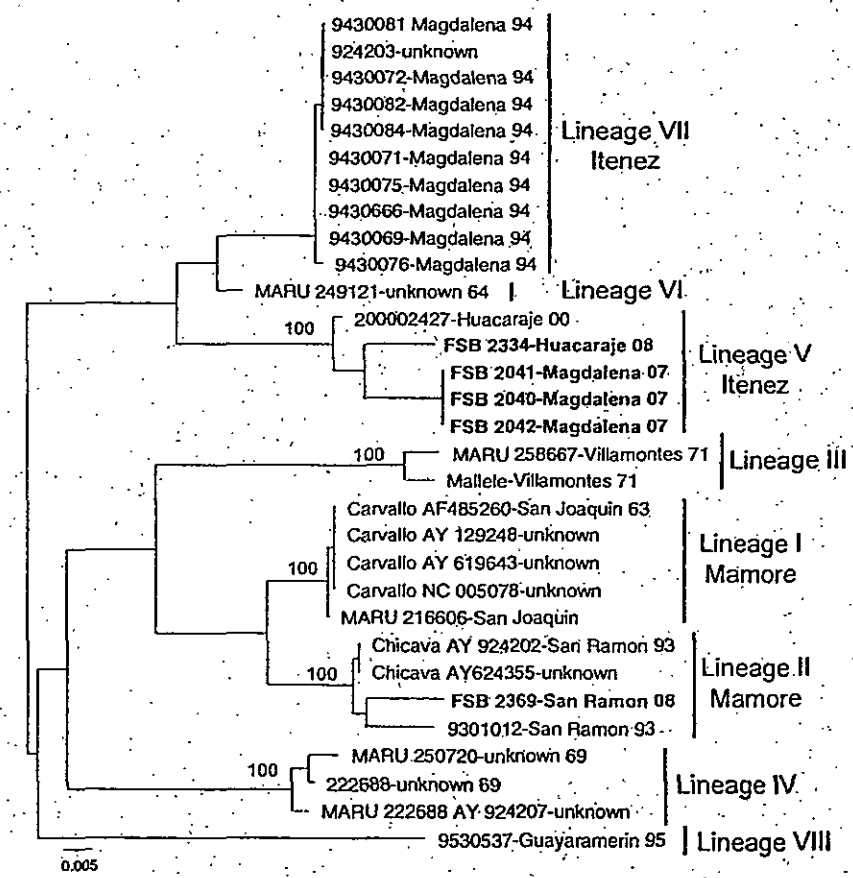

Figure. Neighbor-joining phylogenetic tree of Machupo virus derived from the glycoprotein precursor gene sequence. The neighbor-joining and maximum likelihood analyses yielded similar phylogenetic trees. Boldface indicates 2007–2008 isolates. Numbers indicate bootstrap values for 1,000 replicates. Scale bar indicates nucleotide substitutions per site.

Ramon (Mamore Province) belonged to lineage II. These isolates showed 10% nucleotide difference within the S segment and a 6% amino acid difference within the glycoprotein precursor gene. Similar genetic diversity has been described with Machupo virus and other arenaviruses (*2–4*). Sequences generated were deposited in GenBank (accession nos. FJ696411, FJ696412, FJ696413, FJ696414, and FJ696415).

It is not known whether lineage VII and I viruses continue to circulate or have been replaced by lineage V and II viruses, respectively. This study confirms the long-term maintenance of distinct phylogenetically forms of Machupo virus in a small area within Beni. Although the distribution of the Machupo virus rodent reservoir (*Calomys callosus*) extends beyond the geographic area of the Machupo cases described, factors that limit the endemic distribution of the virus remain unknown. However, population differences among *C. callosus* may account for the natural nidality of BHF (*5*). Studies are needed to fully identify and understand the ecology of the rodent reservoir and Machupo virus transmission.

Machupo virus continues to cause sporadic cases and focal outbreaks of BHF in Bolivia. We describe 5 confirmed human cases (3 fatal) of Machupo virus infection in Beni Department, Bolivia, an area in which BHF is endemic. That all 5 patients were farmers suggests their infections were probably acquired through occupational exposure. Although all the patients received plasma transfusion from patients who had survived BHF infection, 3 patients still died. An early diagnosis and the rapid administration of Machupo immune plasma before the hemorrhagic phase may increase the chance of survival, as has been observed with other arenavirus infections (*6–8*).

Acknowledgments

We thank Roxana Caceda and Juan Sulca for excellent technical assistance and the personnel of the Bolivian Ministry of Health for supporting our febrile illness surveillance study. Local activities were approved by the Ministry of Health of Bolivia and were developed by CENETROP personnel through local coordinators.

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Patricia V. Aguilar, Wilfredo Camargo, Jorge Vargas, Carolina Guevara, Yelin Roca, Vidal Felices, V. Alberto Laguna-Torres, Robert Tesh, Thomas G. Ksiazek, and Tadeusz J. Kochel

Author affiliations: US Naval Medical Research Center Detachment, Lima, Peru (P.V. Aguilar, C. Guevara, V. Felices, V.A. Laguna-Torres, T. Kochel); Centro Nacional de Enfermedades Tropicales, Santa Cruz, Bolivia (J. Vargas, Y. Roca); El Servicio Departamental de Salud, Beni, Bolivia (W. Camargo); University of Texas Medical Branch, Galveston, Texas, USA (R. Tesh); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (T.G. Ksiazek).

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Address for correspondence: Patricia V. Aguilar, US Naval Medical Research Center Detachment, 3230 Lima Pl, Washington, DC 20521-3230, USA; email: patricia.aguilar@med.navy.mil

Relapsing Fever Spirochete in Seabird Tick, Japan

To the Editor: Tick-borne relapsing fever (TBRF) is caused by infection with spirochetes belonging to the genus *Borrelia*. We previously reported a human case of febrile illness suspected to be TBRF on the basis of serologic examination results; the vector most likely was a genus *Carios* tick that had fed on a seabird colony (*1*). However, surveillance of ticks in the area did not identify *Borrelia* spp. in any of the *Carios* ticks sampled (2). In 2007 and 2008, a borreliosis investigation was conducted on Kutsujima Island (35.71′N, 135.44′E) because

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研究報告の概要

○慢性疲労症候群患者の血液細胞における感染性レトロウイルスXMRVの検出
慢性疲労症候群(CFS)は原因不明の疾患で、全世界に1700万人の患者がいると推定されている。CFS患者の末梢血単核細胞(PBMCs)を検討することにより、患者101名中68名(67%)、健常者の対照群218名中8名(3.7%)において、ヒトガンマレトロウイルスの一種であるxenotropic murine leukemia virus-related virus(XMRV)のDNAを同定した。細胞培養試験では、患者由来XMRVに感染性があり、細胞結合性感染、無細胞性感染のいずれも起こりうることが判明した。CFS患者由来の活性化PBMCs、B細胞、T細胞、血漿への暴露後に、非感染初代リンパ球と指標細胞株において二次感染が成立した。これらの知見はXMRVがCFSの病原因子である可能性を提起する。

報告企業の意見	今後の対応
慢性疲労症候群(CFS)患者の血液細胞から感染性レトロウイルスXMRVのDNAが検出され、XMRVがCFSの病原因子である可能性が提起されたとの報告である。	今後も引き続き、新たなウイルス等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

the pathophysiology of chytridiomycosis appears to be disruption to the osmoregulatory functioning of the skin and consequent osmotic imbalance that leads to cardiac standstill.

To test whether treating electrolyte abnormalities would reduce the clinical signs of disease, we administered an oral electrolyte supplement to *L. caerulea* in the terminal stages of infection, when they lost the righting reflex and could no longer correct their body positions (*26*). Frogs under treatment recovered a normal posture and became more active; one individual recovered sufficiently to climb out of the water onto the container walls, and two individuals were able to jump to avoid capture. These signs of recovery were not observed in any untreated frogs. In addition, treated frogs lived >20 hours longer than untreated frogs [mean time after treatment ± SEM: treated frogs (N = 9), 32 ± 2.8 hours; control frogs (N = 6), 10.7 ± 2.2 hours; Student's t test, $P < 0.001$]. All treated frogs continued to shed skin and ultimately died from the infection, as expected. It is unlikely that electrolyte treatment could prevent death unless the epidermal damage caused by *Bd* is reversed. Although amphibians can generally tolerate greater electrolyte fluctuations than other terrestrial vertebrates (*18*), we suggest that depletion of electrolytes, especially potassium, is important in the pathophysiology of chytridiomycosis. Amphibian plasma potassium concentrations are maintained at constant levels across seasons (*27*), and even moderate hypokalemia is dangerous in humans (*28*).

Our results support the epidermal dysfunction hypothesis, which suggests that *Bd* disrupts cutaneous osmoregulatory function, leading to electrolyte imbalance and death. The ability of *Bd* to compromise the epidermis explains how a superficial skin fungus can be fatal to many species of amphibians; their existence depends on the physiological interactions of the skin with the external environment (*16*–*19*). Disease outbreaks capable of causing population declines require the alignment of multiple variables, including a life-compromising pathophysiology (*1*). Resolving the pathogenesis of chytridiomycosis is a key step in understanding this unparalleled pandemic.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5952/582/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
Tables S1 and S2
References

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Detection of an Infectious Retrovirus, XMRV, in Blood Cells of Patients with Chronic Fatigue Syndrome

Vincent C. Lombardi,[1*] Francis W. Ruscetti,[2*] Jaydip Das Gupta,[3] Max A. Pfost,[1] Kathryn S. Hagen,[1] Daniel L. Peterson,[1] Sandra K. Ruscetti,[4] Rachel K. Bagni,[5] Cari Petrow-Sadowski,[6] Bert Gold,[2] Michael Dean,[2] Robert H. Silverman,[3] Judy A. Mikovits[1]†

Chronic fatigue syndrome (CFS) is a debilitating disease of unknown etiology that is estimated to affect 17 million people worldwide. Studying peripheral blood mononuclear cells (PBMCs) from CFS patients, we identified DNA from a human gammaretrovirus, xenotropic murine leukemia virus–related virus (XMRV), in 68 of 101 patients (67%) as compared to 8 of 218 (3.7%) healthy controls. Cell culture experiments revealed that patient-derived XMRV is infectious and that both cell-associated and cell-free transmission of the virus are possible. Secondary viral infections were established in uninfected primary lymphocytes and indicator cell lines after their exposure to activated PBMCs, B cells, T cells, or plasma derived from CFS patients. These findings raise the possibility that XMRV may be a contributing factor in the pathogenesis of CFS.

Chronic fatigue syndrome (CFS) is a disorder of unknown etiology that affects multiple organ systems in the body. Patients with CFS display abnormalities in immune system function, often including chronic activation of the innate immune system and a deficiency in natural killer cell activity (*1*, *2*). A number of viruses, including ubiquitous herpesviruses and enteroviruses, have been implicated as possible environmental triggers of CFS (*1*). Patients with CFS often have active β herpesvirus infections, suggesting an underlying immune deficiency.

The recent discovery of a gammaretrovirus, xenotropic murine leukemia virus–related virus (XMRV), in the tumor tissue of a subset of prostate cancer patients prompted us to test whether XMRV might be associated with CFS. Both of these disorders, XMRV-positive prostate cancer and CFS, have been linked to alterations in the antiviral enzyme RNase L (*3*–*5*). Using the Whittemore Peterson Institute's (WPI's) national

[1]Whittemore Peterson Institute, Reno, NV 89557, USA. [2]Laboratory of Experimental Immunology, National Cancer Institute–Frederick, Frederick, MD 21701, USA. [3]Department of Cancer Biology, The Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA. [4]Laboratory of Cancer Prevention, National Cancer Institute–Frederick, Frederick, MD 21701, USA. [5]Advanced Technology Program, National Cancer Institute–Frederick, Frederick, MD 21701, USA. [6]Basic Research Program, Scientific Applications International Corporation, National Cancer Institute–Frederick, Frederick, MD 21701, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: judym@wpinstitute.org

tissue repository, which contains samples from well-characterized cohorts of CFS patients, we isolated nucleic acids from PBMCs and assayed the samples for XMRV *gag* sequences by nested polymerase chain reaction (PCR) (*5*, *6*). Of the 101 CFS samples analyzed, 68 (67%) contained XMRV *gag* sequence. Detection of XMRV was confirmed in 7 of 11 WPI CFS samples at the Cleveland Clinic by PCR-amplifying and sequencing segments of XMRV *env* [352 nucleotides (nt)] and *gag* (736 nt) in CFS PBMC DNA (Fig. 1A) (*6*). In contrast, XMRV *gag* sequences were detected in 8 of 218 (3.7%) PBMC DNA specimens from healthy individuals. Of the 11 healthy control DNA samples analyzed by PCR for both *env* and *gag*, only one sample was positive for *gag* and none for *env* (Fig. 1B). In all positive cases, the XMRV *gag* and *env* sequences were more than 99% similar to those previously reported for prostate tumor–associated strains of XMRV (VP62, VP35, and VP42) (fig. S1) (*5*).

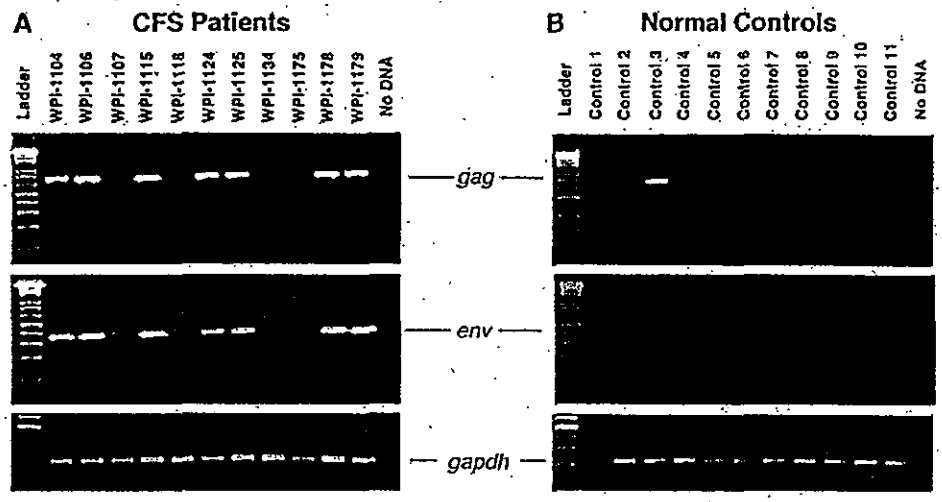

Fig. 1. XMRV sequences in PBMC DNA from CFS patients. Single-round PCR results for *gag*, *env*, and *gapdh* sequences in PBMCs of (**A**) CFS patients and (**B**) healthy controls are shown. The positions of the amplicons are indicated and DNA markers (ladder) are shown. These are representative results from one group of 20 patients.

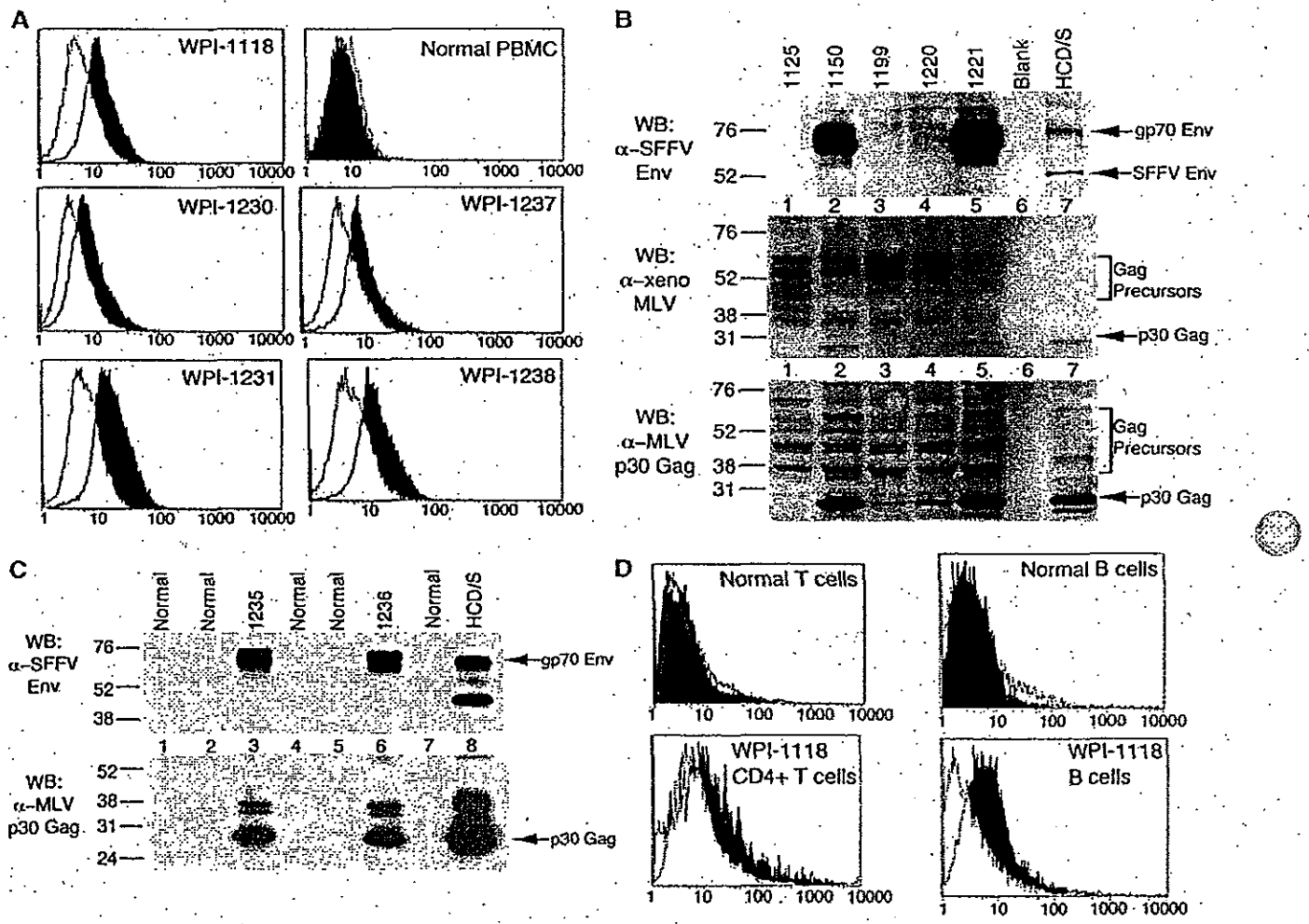

Fig. 2. Expression of XMRV proteins in PBMCs from CFS patients. (**A**) PBMCs were activated with phytohemagglutinin and interleukin-2, reacted with a mAb to MLV p30 Gag, and analyzed by IFC. (**B**) Lysates of activated PBMCs from CFS patients (lanes 1 to 5) were analyzed by Western blots with rat mAb to SFFV Env (top panel), goat antiserum to xenotropic MLV (middle panel), or goat antiserum to MLV p30 Gag (bottom panel). Lane 7, lysate from SFFV-infected HCD-57 cells. Molecular weight markers in kilodaltons are at left. (**C**) Lysates of activated PBMCs from healthy donors (lanes 1, 2, 4, 5, and 7) or from CFS patients (lanes 3 and 6) were analyzed by Western blots using rat mAb to SFFV Env (top panel) or goat antiserum to MLV p30 Gag (bottom panel). Lane 8, SFFV-infected HCD-57 cells. Molecular weight (MW) markers in kilodaltons are at left. (**D**) $CD4^+$ T cells (left) or $CD19^+$ B cells (right) were purified, activated, and examined by flow cytometry for XMRV Gag with a mAb to MLV p30 Gag.

Sequences of full-length XMRV genomes from two CFS patients and a partial genome from a third patient were generated (table S1). CFS XMRV strains 1106 and 1178 each differed by 6 nt from the reference prostate cancer strain XMRV VP62 (EF185282), and with the exception of 1 nt, the variant nucleotides mapped to different locations within the XMRV genome, suggesting independent infections. In comparison, prostate cancer–derived XMRV strains VP35 and VP42 differed from VP62 by 13 and 10 nt, respectively. Thus, the complete XMRV genomes in these CFS patients

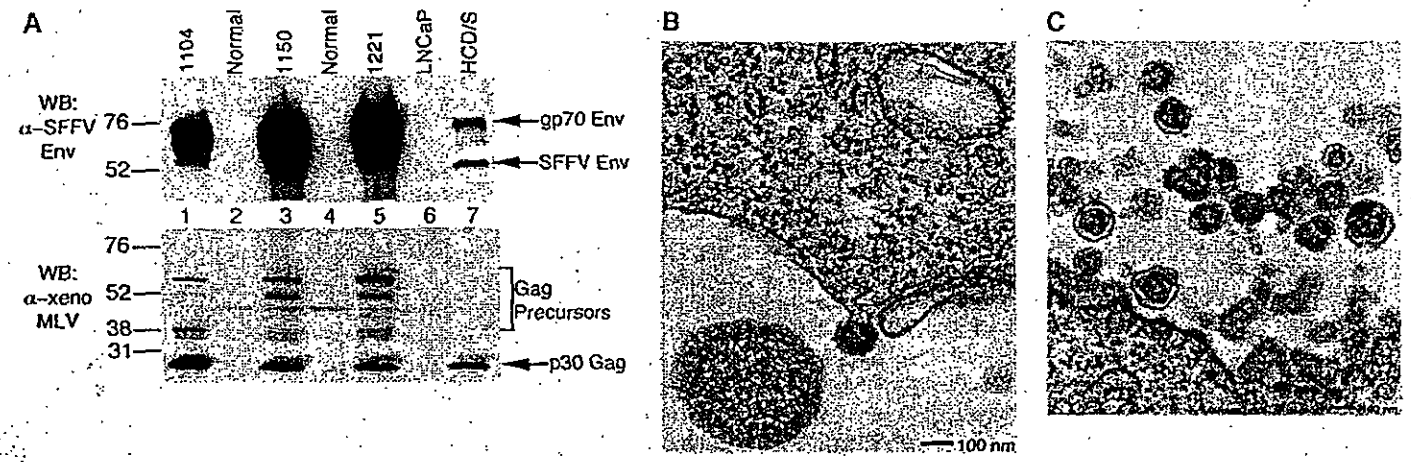

Fig. 3. Infectious XMRV in PBMCs from CFS patients. (**A**) Lysates of LNCaP cells cocultured with PBMCs from CFS patients (lanes 1, 3, and 5) or healthy donors (lanes 2 and 4) were analyzed by Western blots with rat mAb to SFFV Env (top panel) or goat antiserum to xenotropic MLV (bottom panel). Lane 6, uninfected LNCaP; lane 7, SFFV-infected HCD-57 cells. MW markers in kilodaltons are at left. (**B**) Transmission electron micrograph of a budding viral particle from LNCaP cells infected by incubation with an activated T cell culture from a CFS patient. (**C**) Transmission electron micrograph of virus particles released by infected LNCaP cells.

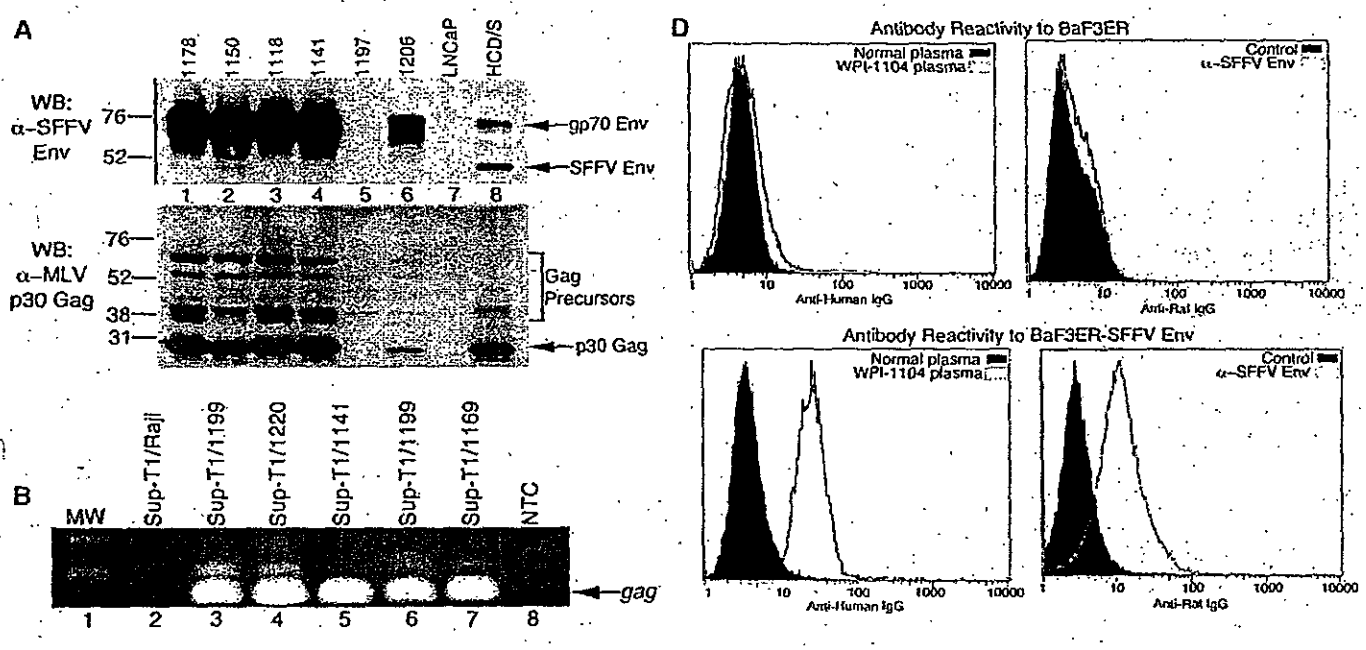

Fig. 4. Infectious XMRV and antibodies to XMRV in CFS patient plasma. (**A**) Plasma from CFS patients (lanes 1 to 6) were incubated with LNCaP cells and lysates were prepared after six passages. Viral protein expression was detected by Western blots with rat mAb to SFFV Env (top panel) or goat antiserum to MLV p30 Gag (bottom panel). Lane 7, uninfected LNCaP; lane 8, SFFV-infected HCD-57 cells. MW markers in kilodaltons are at left. (**B**) Cell-free transmission of XMRV to the SupT1 cell line was demonstrated using transwell coculture with patient PBMCs, followed by nested *gag* PCR. Lane 1, MW marker. Lane 2, SupT1 cocultured with Raji. Lanes 3 to 7, SupT1 cocultured with CFS patient PBMCs. Lane 8, no template control (NTC). (**C**) Normal T cells were exposed to cell-free supernatants obtained from T cells (lanes 1, 5, and 6) or B cells (lane 4) from CFS patients. Lanes 7 and 8 are secondary infections of normal activated T cells. Initially, uninfected primary T cells were exposed to supernatants from PBMCs of patients WPI-1220 (lane 7) and WPI-1221 (lane 8). Lanes 2 and 3, uninfected T cells; lane 9, SFFV-infected HCD-57 cells. Viral protein expression was detected by Western blot with a rat mAb to SFFV Env. MW markers in kilodaltons are at left. (**D**) Plasma samples from a CFS patient or from a healthy control as well as SFFV Env mAb or control were reacted with BaF3ER cells (top) or BaF3ER cells expressing recombinant SFFV Env (bottom) and analyzed by flow cytometry. IgG, immunoglobulin G.

were >99% identical in sequence to those detected in patients with prostate cancer. To exclude the possibility that we were detecting a murine leukemia virus (MLV) laboratory contaminant, we determined the phylogenetic relationship among endogenous (non-ecotropic) MLV sequences, XMRV sequences, and sequences from CFS patients 1104, 1106, and 1178 (fig. S2). XMRV sequences from the CFS patients clustered with the XMRV sequences from prostate cancer cases and formed a branch distinct from non-ecotropic MLVs common in inbred mouse strains. Thus, the virus detected in the CFS patients' blood samples is unlikely to be a contaminant.

To determine whether XMRV proteins were expressed in PBMCs from CFS patients, we developed intracellular flow cytometry (IFC) and Western blot assays, using antibodies (Abs) with novel viral specificities. These antibodies included, among others, (i) rat monoclonal antibody (mAb) to the spleen focus-forming virus (SFFV) envelope (Env), which reacts with all polytropic and xenotropic MLVs (*7*); (ii) goat antisera to whole mouse NZB xenotropic MLV; and (iii) a rat mAb to MLV p30 Gag (*8*). All of these Abs detected the human VP62 XMRV strain grown in human Raji, LNCaP, and Sup-T1 cells (fig. S3) (*5*). IFC of activated lymphocytes (*6*, *9*) revealed that 19 of 30 PBMC samples from CFS patients reacted with the mAb to MLV p30 Gag (Fig. 2A). The majority of the 19 positive samples also reacted with antisera to other purified MLV proteins (fig. S4A). In contrast, 16 healthy control PBMC cultures tested negative (Fig. 2A and fig. S4A). These results were confirmed by Western blots (Fig. 2, B and C) (*6*) using Abs to SFFV Env, mouse xenotropic MLV, and MLV p30 Gag. Samples from five healthy donors exhibited no expression of XMRV proteins (Fig. 2C). The frequencies of CFS cases versus healthy controls that were positive and negative for XMRV sequences were used to calculate a Pearson χ^2 value of 154 (two-tailed P value of 8.1×10^{-35}). These data yield an odds ratio of 54.1 (a 95% confidence interval of 23.8 to 122), suggesting a nonrandom association with XMRV and CFS patients.

To determine which types of lymphocytes in blood express XMRV, we isolated B and T cells from one patient's PBMCs (*6*). Using mAb to MLV p30 Gag and IFC, we found that both activated T and B cells were infected with XMRV (Fig. 2D and fig. S4A). Furthermore, using mAb to SFFV Env, we found that >95% of the cells in a B cell line developed from another patient were positive for XMRV Env (fig. S4B). XMRV protein expression in CFS patient–derived activated T and B cells grown for 42 days in culture was confirmed by Western blots (fig. S4C) using Abs to SFFV Env and xenotropic MLV.

We next investigated whether the viral proteins detected in PBMCs from CFS patients represent infectious XMRV. Activated lymphocytes (*6*) were cocultured with LNCaP, a prostate cancer cell line with defects in both the JAK-STAT and RNase L pathways (*10*, *11*) that was previously shown to be permissive for XMRV infection (*12*). After coculture with activated PBMCs from CFS patients, LNCaP cells expressed XMRV Env and multiple XMRV Gag proteins when analyzed by Western blot (Fig. 3A) and IFC (fig. S5A). Transmission electron microscopy (EM) of the infected LNCaP cells (Fig. 3B), as well as virus preparations from these cells (Fig. 3C), revealed 90- to 100-nm-diameter budding particles consistent with a gamma (type C) retrovirus (*13*).

We also found that XMRV could be transmitted from CFS patient plasma to LNCaP cells when we applied a virus centrifugation protocol to enhance infectivity (*6*, *14*, *15*). Both XMRV gp70 Env and p30 Gag were abundantly expressed in LNCaP cells incubated with plasma samples from 10 of 12 CFS patients, whereas no viral protein expression was detected in LNCaP cells incubated with plasma samples from 12 healthy donors (Fig. 4A). Likewise, LNCaP cells incubated with patient plasma tested positive for XMRV p30 Gag in IFC assays (fig. S5B). We also observed cell-free transmission of XMRV from the PBMCs of CFS patients to the T cell line SupT1 (Fig. 4B) and both primary and secondary transmission of cell-free virus from the activated T cells of CFS patients to normal T cell cultures (Fig. 4C). Together, these results suggest that both cell-associated and cell-free transmission of CFS-associated XMRV are possible.

We next investigated whether XMRV stimulates an immune response in CFS patients. For this purpose, we developed a flow cytometry assay that allowed us to detect Abs to XMRV Env by exploiting its close homology to SFFV Env (*16*). Plasma from 9 out of 18 CFS patients infected with XMRV reacted with a mouse B cell line expressing recombinant SFFV Env (BaF3ER-SFFV-Env) but not to SFFV Env negative control cells (BaF3ER), analogous to the binding of the SFFV Env mAb to these cells (Fig. 4D and S6A). In contrast, plasma from seven healthy donors did not react (Fig. 4D and fig. S6A). Furthermore, all nine positive plasma samples from CFS patients but none of the plasma samples from healthy donors blocked the binding of the SFFV Env mAb to SFFV Env on the cell surface (fig. S6B). These results are consistent with the hypothesis that CFS patients mount a specific immune response to XMRV.

Neurological maladies and immune dysfunction with inflammatory cytokine and chemokine up-regulation are some of the most commonly reported features associated with CFS. Several retroviruses, including the MLVs and the primate retroviruses HIV and HTLV-1, are associated with neurological diseases as well as cancer (*17*). Studies of retrovirus-induced neurodegeneration in rodent models have indicated that vascular and inflammatory changes mediated by cytokines and chemokines precede the neurological pathology (*18*, *19*). The presence of infectious XMRV in lymphocytes may account for some of these observations of altered immune responsiveness and neurological function in CFS patients.

We have discovered a highly significant association between the XMRV retrovirus and CFS. This observation raises several important questions. Is XMRV infection a causal factor in the pathogenesis of CFS or a passenger virus in the immunosuppressed CFS patient population? What is the relationship between XMRV infection status and the presence or absence of other viruses that are often associated with CFS (e.g., herpesviruses)? Conceivably these viruses could be cofactors in pathogenesis, as is the case for HIV-mediated disease, in which co-infecting pathogens play an important role (*20*). Patients with CFS have an elevated incidence of cancer (*21*). Does XMRV infection alter the risk of cancer development in CFS? As noted above, XMRV has been detected in prostate tumors from patients expressing a specific genetic variant of the *RNASEL* gene (*5*). In contrast, in our study of this CFS cohort, we found that XMRV infection status does not correlate with the *RNASEL* genotype (*6*) (table S2).

Finally, it is worth noting that 3.7% of the healthy donors in our study tested positive for XMRV sequences. This suggests that several million Americans may be infected with a retrovirus of as yet unknown pathogenic potential.

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Note added in proof: V.C.L. is operations manager of Viral Immune Pathologies Laboratory, which is in negotiations with the Whittemore Peterson Institute to offer a diagnostic test for XMRV.

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Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References

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Complete Reconstitution of a Highly Reducing Iterative Polyketide Synthase

Suzanne M. Ma,[1] Jesse W.-H. Li,[2] Jin W. Choi,[3] Hui Zhou,[1] K. K. Michael Lee,[3] Vijayalakshmi A. Moorthie,[2] Xinkai Xie,[1] James T. Kealey,[4] Nancy A. Da Silva,[3] John C. Vederas,[2]* Yi Tang[1]*

Highly reducing iterative polyketide synthases are large, multifunctional enzymes that make important metabolites in fungi, such as lovastatin, a cholesterol-lowering drug from *Aspergillus terreus*. We report efficient expression of the lovastatin nonaketide synthase (LovB) from an engineered strain of *Saccharomyces cerevisiae*, as well as complete reconstitution of its catalytic function in the presence and absence of cofactors (the reduced form of nicotinamide adenine dinucleotide phosphate and *S*-adenosylmethionine) and its partner enzyme, the enoyl reductase LovC. Our results demonstrate that LovB retains correct intermediates until completion of synthesis of dihydromonacolin L, but off-loads incorrectly processed compounds as pyrones or hydrolytic products. Experiments replacing LovC with analogous MlcG from compactin biosynthesis demonstrate a gate-keeping function for this partner enzyme. This study represents a key step in the understanding of the functions and structures of this family of enzymes.

Nature uses an amazing array of enzymes to make natural products (*1*). Among these metabolites, polyketides represent a class of over 7000 known structures of which more than 20 are commercial drugs (*2*). Among the most interesting but least understood enzymes making these compounds are the highly reducing iterative polyketide synthases (HR-IPKSs) found in filamentous fungi (*3*). In contrast to the well-studied bacterial type I PKSs that operate in an assembly line fashion (*4*), HR-IPKSs are megasynthases that function iteratively by using a set of catalytic domains repeatedly in different combinations to produce structurally diverse fungal metabolites (*5*). One such metabolite is lovastatin, a cholesterol-lowering drug from *Aspergillus terreus* (*6*). This compound is a precursor to simvastatin (Zocor, Merck, Whitehouse Station, NJ), a semi-synthetic drug that had annual sales of more than $4 billion before loss of patent protection in 2006 (*7*).

Biosynthesis of lovastatin proceeds via dihydromonacolin L (acid form 1, lactone form 2), a product made by the HR-IPKS lovastatin nonaketide synthase (LovB), with the assistance of a separate enoyl reductase, LovC (*8*) (Fig. 1). LovB is a 335-kD protein that contains single copies of ketosynthase (KS), malonyl–coenzyme A (CoA) acyltransferase (MAT), dehydratase (DH), methyltransferase (MT), ketoreductase (KR), and acyl-carrier protein (ACP) domains, as well as a section that is homologous to the condensation (CON) domain found in nonribosomal peptide synthetases (NRPSs) (*9*). It also contains a domain that resembles an enoyl reductase (ER) but lacks that activity. LovB must catalyze ~35 reactions and use different permutations of tailoring domains after each of the eight chain-extension steps to yield the nonaketide, dihydromonacolin L (2). This enzyme also catalyzes a biological Diels-Alder reaction during the assembly process to form the decalin ring system (*10*). In vitro studies of LovB (*11*) have been hampered by an inability to obtain sufficient amounts of the functional purified megasynthase from either *A. terreus* or heterologous *Aspergillus* hosts. As a result, the programming that governs metabolite assembly by LovB or other HR-IPKSs is not understood. Key aspects that remain to be elucidated include (i) the catalytic and structural roles of each domain in the megasynthase, (ii) substrate specificities of the catalytic domains and their tolerance to perturbation in megasynthase functions, and (iii) factors governing the choice of different combinations of domains during each iteration of catalysis. To initiate such studies, we engineered an expression system in yeast to produce large amounts of LovB and examined the influence of cofactors and the ER partner (e.g., LovC) on product formation.

The engineered *Saccharomyces cerevisiae* strain BJ5464-NpgA, which contains a chromosomal copy of the *Aspergillus nidulans* phosphopantetheinyl (ppant) transferase gene *npgA* (*12*), was the expression host. A C-terminal hexahistidine-tagged LovB was placed under the control of the *S. cerevisiae ADH2* promoter (*13*, *14*) on an episomal plasmid (YEpLovB-6His). Abundant amounts of the intact LovB could be purified from the soluble fraction to near homogeneity with a final yield of ~4.5 mg/L (fig. S1). We used mass analysis of tryptic digest fragments to verify the identity of the recombinant LovB. The ACP domain of LovB was determined to be nearly completely phosphopantetheinylated by using a ppant ejection assay with high-resolution quadrupole orthogonal acceleration–time-of-flight mass spectrometry (fig. S2). To ascertain activity of the resulting LovB and to examine the necessity for cofactors, malonyl-CoA alone was first added to the purified enzyme in buffer. Whole-cell feeding studies of doubly [^{13}C, ^{2}H]-labeled acetate to cultures of *A. terreus* showed that all three acetate hydrogens were incorporated into the acetate-derived starter units for both the nonaketide and diketide moieties in lovastatin (*15*). The purified LovB can use malonyl-CoA for both chain priming and chain elongation, loading malonate with decarboxylation to make the acetyl starter unit. Although LovB is able to prime with and elongate the chain by two further condensations with malonyl-CoA, in the absence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), no ketoreduction occurs. The dominant product is lactone 3 (Fig. 2A, trace i), which forms by enolization and cyclization with off-loading of the unreduced triketide. Addition of NADPH to this system enables function of the KR domain. In this and subsequent experiments, the malonyl-CoA could be conveniently synthesized in situ by malonyl-CoA synthase (MatB) from *Rhizobium trifolii* using free malonate and CoA (*16*). With KR enabled, LovB makes penta-, hexa-, and heptaketide pyrones 4 to 6, as well as ketones 7 and 8 (Fig. 2A, trace ii). The structures were confirmed by chemical synthesis of authentic standards, except for heptaketide 6, which proved very unstable. However, the mass increase of 26 atomic mass units for 6 and its red shift in the ultraviolet spectrum when compared to 5 are consistent with its proposed heptaketide pyrone structure (table S3). Compounds 7 and 8 result from thioester hydrolysis of penta- and hexaketides stalling on the ACP at the β-keto stage. The resulting β-keto acids spontaneously decarboxylate to afford 7 and 8. Formation of compounds 4 to 8 illustrates that derailment in the normal programmed steps, namely the lack of methylation due to the absence of *S*-adenosylmethionine

[1]Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, CA 90095, USA. [2]Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada. [3]Department of Chemical Engineering and Materials Science, University of California, Irvine, CA 92697, USA. [4]Amyris Biotechnologies, 5885 Hollis Street, Suite 100, Emeryville, CA 94608, USA.
*To whom correspondence should be addressed. E-mail: john.vederas@ualberta.ca (J.C.V.); yitang@ucla.edu (Y.T.)

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研究報告の概要

（目的）日本で採血された血漿由来の静注用免疫グロブリン製剤(IVIG)に Classical Swine Influenza A(H1N1)virus 及び Influenza A(H1N1)pdm virus に反応する抗体が含まれているかを調べ、ドナーが免疫を獲得している可能性について検討した。
（材料と方法）Classical Swine Influenza A(H1N1)virus は「A/Swine/Hokkaido/2/1981」を使用した。Influenza A(H1N1)pdm virus は大阪で分離したウイルスを用いた。IVIG は日本で採血された原料により2008年に製造されたロットを用いた。赤血球凝集抑制能（HI価）は、8HAのウイルス液に等量の希釈サンプルを加え、モルモット赤血球に対する完全凝集制御を示す最大希釈倍数で求めた。中和抗体価（NT活性）は100FFUのウイルスに対して50%以上の感染阻害を示す最大希釈倍数で求めた。
（結果）IVIGにブタ及び新型ウイルスに対するHI及びNT活性が認められ、その値はそれぞれ8倍、64倍であった。
（考察）日本で採血された血漿由来の IVIG にも HI 及び NT 活性が認められることから、日本においてもある程度の率で Influenza A(H1N1)pdm virus に反応する抗体を保有しているドナーが存在すると推測された。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
日本で採血された血漿を原料として製造された静注用免疫グロブリン製剤(IVIG)に新型インフルエンザウイルスに反応する抗体が含まれていたことについての報告である。 インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。

1P073

季節性インフルエンザウイルスに対するヒト免疫グロブリン製剤の抗体価

浦山 健[1,2]、纐纈律子[2]、辻川宗男[1]、柚木幹弘[1,2]、萩原克郎[5]、高橋和郎[4]、奥野良信[3]、生田和良[2]
株式会社ベネシス 研究開発本部 大阪研究所[1]、
大阪大学微生物病研究所 ウイルス免疫分野[2]、
財団法人阪大微生物病研究会 観音寺研究所[3]、
大阪府立公衆衛生研究所[4]、
酪農学園大学獣医学部[5]
✉ Urayama.Takeru@mk.mt-pharma.co.jp

【目的と意義】
インフルエンザワクチンは流行予測に基づいて接種株が決定され、国民に接種されている。このような背景をもつドナー数万人分のプール血漿から製造される静注用免疫グロブリン製剤（IVIG）は、当然ワクチン株に対して高い赤血球凝集抑制能（HI価）を有する。しかしながら、本質的にグロブリンに期待される機能は中和能（NT価）であるにも関わらず、IVIGとNT価に関する知見は少ない。また、IVIGの製造時を起点とし、その前後の時期に分離されたウイルス株への交差反応性に関する知見も少ない。そこで私たちはIVIGに含まれる抗インフルエンザウイルス抗体の特徴を明らかにするため、製造時期及び地域（米国と日本）の異なるIVIG及び分離時期の異なるウイルス株を用いて、HI価及びNT価の比較を行った。
【材料と方法】
製造時期（1999～2008年）または、原料の採漿場所（米国又は日本）が異なる複数のIVIGロットを用いて、各種インフルエンザウイルス株（ワクチン株及び野外分離株）に対するHI価およびNT価を確認した。HI価は、8HAのウイルス液に等量の希釈サンプルを加え、モルモット赤血球に対する完全凝集抑制を示す最大希釈倍数で求めた。NT価は、100FFUのウイルスに対して50%以上の感染阻害を示す最大希釈倍数で求めた。
【結果と考察】
製造時以前に分離され、且つその後ワクチン株として採用されたウイルス株に対して、IVIGは高い抗体価を示した。また、製造時以降新たに分離されたウイルス株に対しても交差反応性を示した。これらの結果からIVIGは製造時期や採漿場所に関わらず、様々な季節性インフルエンザウイルスに反応する抗体を含んでいると考えられた。今後はこの分離時期・地域をまたがって反応する抗体の本質を明らかにする必要がある。

1P074

Classical Swine Influenza A(H1N1)virus及びInfluenza A(H1N1)pdm virusに対する静注用グロブリン製剤の抗体価

井手野祥次[1]、纐纈律子[2]、ドウアナリワ[2]、佐々木正大[2]、浦山 健[1,2]、柚木幹弘[1,2]、萩原克郎[5]、高橋和郎[4]、奥野良信[3]、生田和良[2]
株式会社ベネシス 大阪研究所[1]、
大阪大学微生物病研究所 ウィルス免疫分野[2]、
財団法人阪大微生物病研究会 観音寺研究所[3]、
大阪府立公衆衛生研究所[4]、
酪農学園大学獣医学部[5]
✉ Ideno.Shouji@ma.mt-pharma.co.jp

【目的と意義】
2009年4月は顕在化した新型インフルエンザの世界的流行は想定されていたトリ由来のA/H5N1とは異なり、ブタ由来のA/H1N1であった。今回発生したInfluenza A (H1N1) pdmは抗ウイルス剤（タミフル・リレンザ）に感受性であるが、Seasonal Influenza A (H1N1) virusとは抗原性が異なり、従来の季節型ウイルスワクチンが効かないことが指摘されている。しかしながら、高齢者はこの新型インフルエンザに罹患しにくいという報告もあり、一部のドナーは免疫を獲得していることが考えられた。そこで、私たちは日本で採血された血漿を原料として製造された静注用グロブリン製剤（IVIG）にClassical Swine Influenza A (H1N1) virus及びInfluenza A (H1N1) pdm virusに反応する抗体が含まれているかを調べ、ドナーが免疫を獲得している可能性について検討を行った。
【材料と方法】
Classical Swine Influenza A (H1N1) virusは「A/ Swine/ Hokkaido/2/1981」を使用した。Influenza A (H1N1) pdm virusは大阪で分離されたウイルスを用いた。IVIGは日本で採血された原料により2008年に製造されたロットを用いた。HI価は、8HAのウィルス液に等量の希釈サンプルを加え、モルモット赤血球に対する完全凝集抑制を示す最大希釈倍数で求めた。中和抗体価（NT）は100FFUのウイルスに対して50%以上の感染阻害を示す最大希釈倍数で求めた。
【結果】
IVIGにブタ及び新型ウィルスに対するHI及びNT活性が認められ、その値はそれぞれ8倍、64倍であった。
【考察】
米国における保存血漿のNovel influenza A (H1N1) virusに対する抗体保有率に関する報告が2009年5月22日のMMWRに掲載された。日本で採血された血漿由来のIVIGにもHI及びNT活性が認められることから、日本においてもある程度の率でInfluenza A (H1N1) pdm virusに反応する抗体を保有しているドナーが存在すると推測された。

医薬品
医薬部外品　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2009年11月16日	該当なし	

一般的名称	人ハプトグロビン	研究報告の公表状況	FDA/Vaccines, Blood&Biologicals/2009/11/13	公表国 アメリカ
販売名（企業名）	ハプトグロビン静注2000単位「ベネシス」（ベネシス）			

研究報告の概要

米国FDAによる2009年11月付の業界向けガイダンス（案）「パンデミック（H1N1）2009インフルエンザウイルスへの対応における血液供給の保存，血液製剤の安全性，血液ドナーの適合性に関する推奨」が出された。
示された推奨の内容は以下のとおりである。
A. バックアップ要員の訓練
パンデミック（H1N1）2009インフルエンザウイルスにより引き起こされる疾患の範囲は未知であるので、要員不足を予期し、適正なバックアップ要員を持つことを推奨する。更に、最重要な機能については複数のバックアップ要員を訓練すべきである。バックアップ要員は継続する訓練プログラムで訓練すべきである。
B. 血液ドナー適合性、ドナーの延期そして製剤管理
一般的にドナーの医療歴は採血時に入手される。しかしながら、21 CFR 640.3(a)及び 640.63(a)の下では全血または原料血漿の供給源としてのドナーの適格性は採血日に確立されなければならない。これらの規則は、明確に採血日を定義してない。時々、採血前にドナーに示された質問に対する返答は血液事業者による再調査により不完全であることが発見される。採血から24時間以内に、ドナー歴問診票に対するドナーの返答を明確にするあるいは省略された質問に対する返答を入手する必要がある。
パンデミック（H1N1）2009ウイルスに感染したまたは感染した疑いのあるドナーは、解熱剤の投薬なしで熱が下がり、それ以外の症状もなくなってから、少なくとも24時間は採血を延期しなければならない。
献血後48時間以内にパンデミック（H1N1）2009インフルエンザあるいはインフルエンザ様疾患の可能性のあるドナーについて情報を入手した場合、メディカルディレクターは安全性を評価しなければならない。
C. 承認された申請に対する変更
認定血液事業者としての承認済みの申請について、以下の変更申請を提出してもよい。その試験所がFDAに登録され、ドナー試験を実施しているならば、その外部試験所を使用すること、等。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過膜処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
パンデミック(H1N1)2009インフルエンザウイルスへの対応における血液供給の保存、血液製剤の安全性、血液ドナーの適合性に関す業界向けガイダンス（案）である。 インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。

Guidance for Industry

Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

DRAFT GUIDANCE

This guidance document is for comment purposes only.

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm..

For questions on the content of this guidance contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2009

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Contains Nonbinding Recommendations

Draft – Not for Implementation

Guidance for Industry

Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance document provides recommendations for assessing blood donor suitability and blood product safety and maintaining blood and blood product availability in response to pandemic (H1N1) 2009 virus. It is intended for establishments that manufacture Whole Blood and blood components intended for use in transfusion and blood components intended for further manufacture, including recovered plasma, Source Plasma and Source Leukocytes. Within this guidance, "you" refers to blood establishments; "we" refers to FDA.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

A. Epidemiology and Pathogenesis

The 2009 H1N1 pandemic is caused by a novel influenza A virus of swine origin. On April 26, 2009, then Department of Health and Human Services (DHHS) Acting Secretary Charles E. Johnson, pursuant to section 319 of the Public Health Service Act, 42 U.S.C. § 247d, declared a public health emergency when a novel swine-origin 2009 influenza A (H1N1) virus was identified in California, Texas, Kansas, and New York. The pandemic influenza H1N1 virus has since spread quickly to all fifty states and globally. In June 2009, the World Health Organization (WHO) declared a Phase 6 Level of Pandemic Influenza Alert. This declaration was based upon a standard definition reflecting worldwide spread of the pandemic (H1N1) 2009 virus and the observed

efficiency of human to human transmission. Importantly, a declaration of a pandemic is independent of the severity of illness caused by the virus or the degree of infrastructure disruption. On July 24 2009, DHHS Secretary Kathleen Sebelius renewed DHHS' April 2009 determination that a public health emergency exists nationwide involving pandemic influenza H1N1 that has significant potential to affect national security.

From April 15, 2009 to July 24, 2009, states reported to the Centers for Disease Control and Prevention (CDC) a total of 43,771 confirmed and probable cases of novel influenza A (H1N1) infection. Of these cases reported, 5,011 people were hospitalized and 302 people died.[1,2] From August 30, 2009 to October 24, 2009, 25,985 hospitalizations and 2,916 deaths attributed to influenza and influenza-like illnesses have been reported in the United States (U.S.). CDC has developed a model to estimate the true number of cases in the U.S. The model took the number of cases reported by states and adjusted the figure to account for known sources of underestimation (e.g., not all people with pandemic influenza H1N1 seek medical care, and not all people who seek medical care have specimens collected by their health care providers). Using this approach, it is estimated that more than one million people became infected with novel influenza A (H1N1) between April and June 2009 in the U.S.[3]

The symptoms of human influenza disease caused by pandemic (H1N1) 2009 virus are similar to the symptoms of seasonal flu and include fever, cough, sore throat, runny or stuffy nose, body aches, headache, chills and fatigue. A significant number of people who have been infected with pandemic (H1N1) 2009 virus also have reported diarrhea and vomiting.[4]

The most severe outcomes have been reported among individuals with underlying health problems that are associated with high risk of influenza complications. Pandemic (H1N1) 2009 virus currently remains sensitive to oseltamivir (Tamiflu) and zanamivir (Relenza), though sporadic cases of resistance to oseltamivir have been reported. At this time, there is insufficient information to predict how severe the pandemic (H1N1) 2009 virus outbreak will be in terms of illness and death or infrastructure disruption, or how it will compare with seasonal influenza.

B. Potential Impact of the H1N1 Pandemic on Blood Product Safety and Availability

There is limited information available on pandemic (H1N1) 2009 virus viremia, especially during the asymptomatic period. No case of transfusion transmitted seasonal

[1] http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[2] CDC discontinued reporting of confirmed and probable cases of novel H1N1 infection on July 24, 2009. The most recent total numbers of hospitalizations and deaths due to H1N1 are available on the CDC website. http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[3] http://www.cdc.gov/h1n1flu/surveillanceqa.htm, (Accessed Nov. 2, 2009).
[4] http://www.cdc.gov/h1n1flu/sick.htm, (Accessed Nov. 2, 2009).

influenza has ever been reported in the U.S. or elsewhere, and, to date, no cases of transfusion transmitted pandemic influenza H1N1 have been reported. At this time, the pandemic (H1N1) 2009 virus has not been isolated from blood or serum of asymptomatic, infected individuals; however, studies are ongoing. Furthermore, the potential for transmission of pandemic influenza H1N1 through blood transfusion remains unknown.

In some previous studies, other Influenza A viruses were isolated from blood, and throat secretions or nasopharyngeal mucosa of children with clinical manifestations of influenza (Refs. 1-2). The virus was isolated from blood and throat washings of 1/29 healthy asymptomatic contacts who became ill 12 hours after the specimens were obtained (Ref. 3). From another study, virus isolation was reported from lungs, adrenals and meninges (from autopsy) which indicated that viremia must have been present (Ref. 4). In humans experimentally infected by nasal inoculation, viremia was observed in 4/15 subjects using sensitive culture methods. Symptoms occurred 2 days after initial viremia and one patient remained asymptomatic throughout the study period (22 days) (Ref. 5). However, other investigators were unable to detect viremia in 27 subjects using a similar virus strain and assay methods (Ref. 6).

The pandemic influenza H1N1 virus is a large lipid-enveloped virus. Validation studies performed by product manufacturers have shown that viruses with similar characteristics to the pandemic influenza H1N1 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

Due to its known potential for rapid spread, pandemic (H1N1) 2009 virus has the potential to cause disruptions in the blood supply. A significant number of blood donors, blood establishment staff, and vendors of blood-related supplies (e.g., manufacturers of reagents and blood bags) could be affected as individuals become ill or need to care for ill family members. At the same time, during a widespread outbreak of disease caused by the pandemic (H1N1) 2009 virus, it is anticipated that the demand for blood and blood components may be reduced due to postponement of elective surgery, were that to become necessary in some affected healthcare settings.

In addition, the usual paradigm for ensuring blood availability in response to local disasters (i.e., hurricanes) may not be available under severe pandemic scenarios. In local disasters, interregional transfer of blood from unaffected to affected areas has been an effective strategy. However, in a more severe pandemic scenario, international, national, and regional outbreaks may occur simultaneously and a pandemic wave may last for months. Therefore, advanced planning is reasonable to prepare for the possible need to mitigate the effects of a more severe pandemic and to help ensure that blood is available in affected areas

Standard precautions for avoidance of contact with respiratory secretions may help to reduce the transmission of pandemic (H1N1) 2009 virus in blood and plasma collection establishments. The CDC has issued recommendations for infection control in the

community[5], places of business[6], and in health care settings[7]. CDC also has issued "Interim Infection Control Guidance on 2009 H1N1 Influenza for Personnel at Blood and Plasma Collection Facilities."[8] We recognize the importance of the CDC recommendations for infection control in blood and plasma collection establishments.

III. RECOMMENDATIONS

FDA, in communication with DHHS Office of Public Health and Science, CDC, and the AABB Interorganizational Task Force on Pandemic Influenza and the Blood Supply, monitors blood availability closely. Similarly, we anticipate that you will maintain close communications with your hospital customers to anticipate demand for blood and blood components.

While shortages are not forecast at present, we are reminding you of regulatory pathways and providing regulatory clarification that may be helpful to you both in dealing with the current outbreak and in continuing to stay prepared.

We will continue to review any new scientific information about the potential risk of transfusion transmission of pandemic (H1N1) 2009 virus. We also will monitor closely the impact of the pandemic on blood availability. As our knowledge base grows, we may revise the recommendations in this guidance document as appropriate.

A. Training of Back-Up Personnel

Under 21 CFR 211.25 and 21 CFR 606.20, personnel performing critical functions in blood establishments must be adequate in number, educational background, training and experience, including professional training as necessary, or combination thereof, to assure competent performance of their assigned functions. Given the unknown extent of the disease caused by pandemic (H1N1) 2009 virus, we recommend that you have adequate back-up personnel, in the event of anticipatable personnel shortages. We further recommend that where possible, more than one back-up person should be trained for each critical function. Any such back-up personnel should be trained pursuant to your existing training program. We also recommend that as provided in your training program, you document this training and/or re-training.

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[5] http://www.cdc.gov/h1n1flu/guidance/exclusion.htm, (Accessed Nov. 2, 2009).
[6] http://www.cdc.gov/h1n1flu/business/guidance, (Accessed Nov. 2, 2009).
[7] http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm, (Accessed Nov. 2, 2009).
[8] http://www.cdc.gov/h1n1flu/guidance/blood_facilities.htm.

B. Blood Donor Suitability, Donor Deferral and Product Management

Blood Donor Suitability

In general, a donor medical history is obtained at the time of blood collection. However, under 21 CFR 640.3(a) and 21 CFR 640.63(a), the suitability of a donor as a source of Whole Blood or Source Plasma, must be made on the *day of collection* from the donor. These regulations do not explicitly define the term *day of collection*. Occasionally, donor's responses to the donor questions presented before collection are found to be incomplete upon review by the blood establishment. You may clarify a donor's response to the donor history questionnaire or obtain omitted responses to questions within 24 hours of the collection.

Blood Donor Deferral

- Under current FDA regulations, blood donors must be in good health, as indicated in part by normal temperature and free of acute respiratory diseases on the day of collection (21 CFR 640.3(a), (b)(1) and (4) and 21 CFR 640.63(a), (c)(1) and (7)).

- Available data do not currently support donor deferral for exposure to or contact with a person who has confirmed or probable pandemic (H1N1) 2009 influenza or influenza-like symptoms.

- To ensure donors are in good health on the day of donation as required under 21 CFR 640.3(b) and 21 CFR 640.63(c), donors with a confirmed or probable case of pandemic (H1N1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[9] and they are otherwise asymptomatic.

- Available data do not support the deferral of donors following vaccination with live attenuated influenza vaccines (LAIV) or inactivated influenza vaccines against pandemic (H1N1) 2009 virus or for prophylactic use of the antiviral medications oseltamivir (Tamiflu) and zanamivir (Relenza). However, consistent with the recommendation above, donors taking anti-viral medications for confirmed or probable pandemic (H1N1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[10] and they are otherwise asymptomatic.

[9] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.
[10] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.

Blood Product Management

The recommendations in this section apply to donations of Whole Blood and blood components intended for transfusion. This section does not apply to blood components intended for further manufacture (recovered plasma, Source Plasma, Source Leukocytes) since validation studies have shown that viruses with similar characteristics to pandemic (H1N1) 2009 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

- Upon receipt of post donation information about a donor with confirmed or probable pandemic (H1N1) 2009 disease or influenza like illness within 48 hours after the donation, the Medical Director should evaluate the safety of the previously donated products consistent with existing Standard Operating Procedures (SOPs).

C. Changes to an Approved Application

As provided under 21 CFR 601.12(c)(5), we have determined that the following changes to an approved application for licensed blood establishments may be submitted as a "Supplement-Changes Being Effected".

- Use of a different outside test lab, provided the test lab is registered with FDA and has been performing donor testing.
- Implementation of self-administered donor history questionnaires, provided you follow the critical control points described in FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003), and the submission contains the content recommended for all self-administered procedures and computer assisted interactive procedures outlined in the same guidance.

The recommendations set forth above supersede the recommendations in FDA's "Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture" (July 2001) at section IV.C and FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003) at section IV.A, respectively (in both of these guidances, we previously had determined that these changes would require a "Supplement – Changes Being Effected in 30 Days").

IV. BIOLOGIC PRODUCT DEVIATION AND FATALITY REPORTING

Licensed manufacturers, unlicensed registered blood establishments, and transfusion services are subject to reporting requirements with respect to the reporting of product deviations under

21 CFR 606.171. Blood establishments are not expected to submit biological product deviation reports for post-donation information related to pandemic (H1N1) 2009 virus. If a complication of blood transfusion results in the fatality of a recipient, blood establishments must report the fatality to FDA as soon as possible (21 CFR 606.170(b)).

V. COLLECTION AND USE OF CONVALESCENT PLASMA

Plasma obtained after recovery from an acute infection (convalescent plasma) generally contains highly-specific antibodies directed at the infectious agent, and has theoretical potential to serve as a therapeutic product. In consideration that circumstances could arise where vaccines and antiviral drugs might not be sufficiently available, or where a patient is not responding to approved therapies, transfusion of convalescent plasma has been discussed as a possible empirical treatment during an influenza pandemic. (Ref. 7-8)

In July 2009, the WHO Blood Regulators Network issued a position paper[11] on the collection and use of convalescent plasma as an element in pandemic influenza planning. This paper recommends that scientific studies on the feasibility and medical effectiveness of the collection and use of convalescent plasma, and possibly fractionated immunoglobulins, should be explored through clinical trials. FDA encourages the development of new, safe and effective therapies for influenza. Because of its experimental nature, collection and administration of convalescent plasma should be conducted only under an Investigational New Drug Application. Blood establishments that intend to manufacture convalescent plasma should contact FDA to discuss their plans.

VI. IMPLEMENTATION

This guidance has been issued for comment purposes only.

[11] http://www.who.int/bloodproducts/brn/BRNPosition-ConvPlasma10July09.pdf, (Accessed Nov. 2, 2009).

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医薬品
医薬部外品　　研究報告　調査報告書
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研究報告の概要

多くの生物種に影響を及ぼしている致死的な神経組織障害である伝染性海綿状脳症（TSEs）において、病原性の重要因子は宿主にコードされている正常型プリオン蛋白（PrP^C）の異常型アイソフォーム（PrP^{Sc}）の蓄積である。PrP^CからPrP^{Sc}への変換についての正確な機序は理解されていないが、宿主PrP^C発現はプリオン伝播に効果的な感染性プリオンの産生の必要条件であることは明らかである。哺乳類のTSEsについては多くの研究が実施されているが、魚におけるTSEの病原性についてほとんどわかっていない。
ここで私達は、BSE感染ウシ又はスクレイピー感染ヒツジより調製された脳ホモジネートを経口投与されたヨーロッパヘダイは臨床症状を現わさなかったが、投与2年後に採取されたヨーロッパヘダイの脳は神経変性の徴候と抗タイPrP抗体が上昇し陽性に反応する沈着物の蓄積を示したことを提示する。
非感染動物由来の脳を投与されたコントロール群はそのような徴候を示さなかった。
注目すべきことに、TSE感染脳ホモジネートよりもBSE感染物質を投与された魚に多数のプロテアーゼK抵抗性の沈着物が急速かつ広範囲に現れた。これらのプラーク様凝集はコンゴ好染性と偏光下における複屈折を示し、アミロイド様成分と一致した。プリオン、特にBSEを投与された魚の脳における神経変性と異常な沈着物は公衆衛生上の潜在的リスクに関する懸念を増大させる。
魚の養殖はヒトや他の動物種に対する高タンパク栄養源を供給する経済的で重要な産業であり、感染性哺乳動物PrP^{Sc}に汚染されている養殖魚におけるプリオン病発生の予測は気がかりであり、更なる評価が必要である。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンIH静注250単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
BSE感染ウシ及びスクレイピー感染ヒツジの脳ホモジネートを経口投与されたヨーロッパヘダイの脳は神経変性と抗タイPrP抗体に反応する沈着物の蓄積を示し、公衆衛生上の潜在的リスクに関する懸念を増大させる可能性があるとする報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Evaluation of the Possible Transmission of BSE and Scrapie to Gilthead Sea Bream (*Sparus aurata*)

Evgenia Salta[1☯], Cynthia Panagiotidis[2☯], Konstantinos Teliousis[3], Spyros Petrakis[1,4], Eleftherios Eleftheriadis[5], Fotis Arapoglou[5], Nikolaos Grigoriadis[6], Anna Nicolaou[7], Eleni Kaldrymidou[3], Grigorios Krey[5], Theodoros Sklaviadis[2*]

1 Department of Pharmacology, Aristotle University of Thessaloniki, Thessaloniki, Greece, 2 Centre for Research and Technology-Hellas, Institute of Agrobiotechnology, Thessaloniki, Greece, 3 Laboratory of Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, 4 Max Delbruck Center for Molecular Medicine, Department of Neuroproteomics, Berlin-Buch, Germany, 5 National Agricultural Research Foundation, Fisheries Research Institute, Nea Peramos, Greece, 6 B' Department of Neurology, AHEPA University Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece, 7 Department of Business Administration, University of Macedonia, Thessaloniki, Greece

Abstract

Transmissible spongiform encephalopathies (TSEs), a group of fatal neurodegenerative disorders affecting many species, the key event in disease pathogenesis is the accumulation of an abnormal conformational isoform (PrP[Sc]) of the host-encoded cellular prion protein (PrP[C]). While the precise mechanism of PrP[C] to PrP[Sc] conversion is not understood, it is clear that host PrP[C] expression is a prerequisite for efficient TSE propagation. Although there have been many studies on TSEs in mammalian species, little is known about TSE pathogenesis in fish. Here we show that while gilthead sea bream (*Sparus aurata*) orally challenged with brain homogenates prepared either from a BSE-infected cow or from scrapie-infected sheep developed no clinical prion disease, the brains of TSE-fed fish sampled two years after challenge did show signs of neurodegeneration and accumulation of deposits that reacted positively with antibodies raised against sea bream PrP. The control groups fed with brain from uninfected animals showed no such signs. Remarkably, the deposits developed much more rapidly and extensively in fish challenged with BSE-infected material than in the ones challenged with the scrapie-infected brain homogenate, with numerous deposits being proteinase K-resistant. These plaque-like aggregates exhibited congophilia and green birefringence in polarized light consistent with an amyloid-like component. The neurodegeneration and abnormal deposition in the brains of fish challenged with prions, especially BSE, raises concerns about the potential risk to public health. As fish are a cornerstone in economically important industry providing high protein nutrition for humans and other mammalian species, the prospect of farmed fish being co-farmed with infectious mammalian PrP[Sc], or of a prion disease developing in farmed fish is alarming and requires further evaluation.

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* E-mail: sklaviad@auth.gr

☯ These authors contributed equally to this work.

Introduction

Transmissible spongiform encephalopathies or prion diseases are a group of fatal neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Fatal Familial Insomnia (FFI) and Gerstmann-Sträussler-Scheinker disease (GSS) in humans, scrapie in sheep and goats and bovine spongiform encephalopathy (BSE) in cattle [1].

The transmission of clinical prion diseases is limited by the so-called "species barrier" to conversion of endogenous host prion protein (PrP^{C}) to its abnormal, partially proteinase K-resistant conformational isoform, PrP^{Sc}. When high enough, this "barrier" can greatly impair or prevent potential interspecies transmissions, even under optimal conditions of dose and infection route. However, evidence of TSE replication without accompanying symptoms of clinical disease has prompted debate on the existence of asymptomatic infected individuals in an exposed population [2,3].

The identification of apparent PrP orthologues in lower vertebrates, including fish [4–16], raises the question of their susceptibility to prion diseases. While fish PrP-like sequences do not share high homology with their mammalian relatives (Table S1), they do contain several strongly conserved prion protein structural motifs [17]. Although mammalian to fish TSE transmission is considered unlikely [18], it is not certain that the species barrier would be high enough to prevent TSE transmission to fish.

The BSE epidemic has been linked to TSE-infected cattle feed [19] and the recognition of BSE in domestic cattle inevitably raised concerns about the potential risk to other ruminant and

non-ruminant livestock [20]. The European Commission's TSE risk-reducing measures include a total EU-wide ban on the use of all processed animal protein in livestock and aquaculture feeds. Any consideration of lifting this ban requires a scientific assessment of the TSE transmission risk through fishmeal. Another issue to be addressed is the rising concern that pigs, poultry or fish bred for human consumption and inadvertently fed with TSE-contaminated feed could eventually either develop clinical TSE or serve as reservoirs of infectivity without ever displaying clinical disease themselves. Such an assessment should consider the risk from TSE-contaminated feed being fed to farmed fish [18,21]. In aquaculture, a rapidly growing industry of economic importance in several EU countries, the farmed fish receive commercial feed containing 40–55% protein during the 12–20 months they generally spend in aquaculture facilities. Although remote, the possibility that some of this feed might be contaminated with mammalian prion cannot be excluded.

In the present work, we evaluated the potential transmission of TSEs to gilthead sea bream, a commercially important fish species. After force-feeding with multiple doses of brain homogenate prepared from either healthy or naturally BSE- or scrapie-infected cow or sheep, the fish were monitored for 2 years for evidence of disease development by clinical, histopathological and immunohistochemical criteria. None of the fish examined, showed symptoms of clinical disease. However, signs of neurodegeneration were often present and abnormal deposition was detected in the brains of both the scrapie-challenged and the BSE-challenged fish by 24 months post inoculation.

Results and Discussion

To evaluate the clinical state of the fish, we monitored control and TSE–challenged populations on a daily basis. Since locomotor deficits are often a major feature of the clinical presentation of prion diseases in a variety of hosts, we used the swimming behavior of the challenged fish as an indicator of their general activity and exploratory behavior. No clinical symptoms, including erratic swimming or behavioral abnormalities, were observed in any of the groups monitored. Although unusual in prion disease, a similar absence of clinical symptoms upon interspecies challenge has been reported for both the first passage of sheep scrapie and hamster prion transmission to mice [2]. In these cases, subsequent passage of brain material from the challenged individuals to additional mice did produce clinical disease, thereby demonstrating that asymptomatic animals can harbour high levels of infectious prions in their brains. Additionally, it is important to note that while certain experimentally or virally induced neurodegenerative effects do modify swimming parameters in fish, such as the swimming distance and orientation, the mean velocity, the turning angle and the equilibrium [22–24], this is not always the case. For instance, while both sea bass and sea bream can be infected with nodavirus, a naturally occurring piscine virus that causes brain lesions in both species, sea bream, in contrast to sea bass, show no clinical symptoms of disease [25,26].

To facilitate our evaluation we generated polyclonal antibodies against four different fish PrPs. The specificity of each antiserum was confirmed by both western blot (Fig. S1A) and immunohistochemistry (IHC) (Fig. S2A–C) with normal sea bream brain. Furthermore, anti-mammalian PrP antibodies (6H4, 12F10) did not stain sea bream brain, nor did our anti-fish PrP antisera recognize mammalian PrP^{Sc} (Fig. S1B). Moreover, absorption of our SaurPrP1 (*Sparus aurata* PrP1) antisera with recombinant sea bream PrP-1 protein [6], against which it was raised, resulted in a complete loss of its specific immunostaining in control fish (Fig. S2D).

Initially, in order to determine the distribution of normal endogenous PrP in the central nervous system of gilthead sea bream, we used our polyclonal antisera to perform a detailed immunohistochemical evaluation on brain sections from control fish. Regions displaying abundant PrP included the optic tectum (Fig. 1A), valvula cerebelli (Fig. 1B) and corpus cerebelli (Fig. 1C), while strong PrP-immunopositivity was generally observed in the nerve fibers (Fig. 1D). The most prominently stained regions of the optic tectum, homologue to the superior colliculus in mammals [22], were striatum fibrosum marginale (Fig. 1A and Fig. S2A, B) and striatum fibrosum profundum (Fig. S2A, B). Less intense labeling was observed in the striatum griseum centrale and striatum plexiform fibrosum externum layers of the optic tectum (Fig. 1A and Fig. S2A, B). Cerebellar PrP-immunopositivity was detected mainly in the molecular layer, between Purkinje cell dendrites, and in the granular layer, in the matrix surrounding the granule cells (Fig. 1C). The valvula cerebelli, a rostral protrusion of the cerebellum in the midbrain ventricle that has no counterpart in mammals, showed significant PrP-immunopositivity, similar to that observed in the molecular and granular layers of cerebellum (Fig. 1B and Fig. S2A, B). In cerebral regions, including thalamus, medulla oblongata, diencephalon and the lateral telencephalic pallium, proposed to be a homologue of the mammalian hippocampus [22], we observed intense labeling of fiber bundles (Fig. 1D), consisting primarily of dendritic and axonal prolongations in the neuropil. The same general PrP^{C} expression pattern was observed in challenged and control populations, with no variation detected over time. The remarkable similarity of the overall immunolabeling pattern obtained with our SaurPrP1 antiserum in sea bream brain to the PrP-immunostaining profile in the mammalian brain [27,28] provided further assurance of the specificity of our antibody for piscine PrP.

At the intracellular level, staining outlining the neuronal body was present in most of the neuronal populations observed, e.g. in the large neurons of the brainstem (Figure 1E). Axons displayed intense staining, while diffuse staining was observed inside some of these neuronal somata, suggesting a degree of PrP-immunopositivity within cell departments, e.g. the Golgi complex (Fig. 1E). These findings suggest that the intracellular localization of PrP in fish brain is comparable to the neuronal intracellular localization of mammalian PrP [27,29].

To test for pathology in selected peripheral tissues, we examined intestines and spleens from TSE-challenged fish, sampled at different timepoints. No lesions or any other abnormalities were revealed in comparison to the control individuals. Intestinal PrP-immunoreactivity was evident in the serosa, myenteric plexus and submucous plexus (Fig. 1F). At all timepoints, PrP-immunolabeling in spleen and intestinal tissue was similar in both TSE-challenged and control fish and revealed no PK-resistance and no lesions or any other abnormalities.

Detailed examination of brain sections revealed no histopathological evidence of disease in scrapie–challenged sea bream through 18 months post inoculation (p.i.). At 24 months, however, 2 out of 5 fish showed limited abnormal, PrP-immunoreactive, PK-sensitive, extracellular deposits (Table S3B) in the neuropil of brainstem, diencephalon, corpus cerebelli, valvula cerebelli, optic tectum and telencephalon (Fig. 2). Whilst the number of animals where plaques were found was too small to reach statistical significance, based on the high likelihood that the fish examined developed no plaques, we believe that the observation of aggregates in 2 out of 5 fish at the final time point could be considered as an important event of qualitative (and not

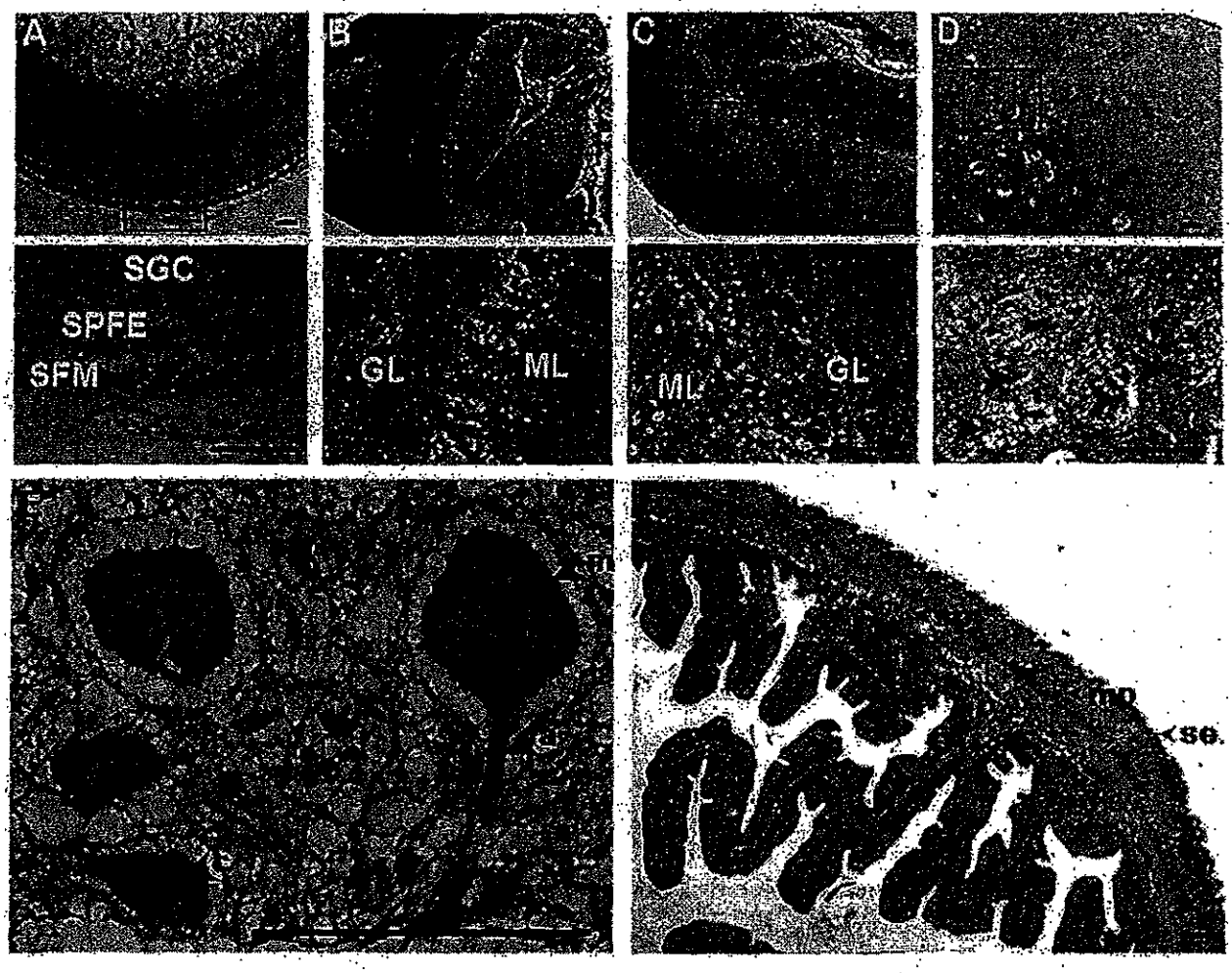

Figure 1. Normal PrP^C distribution in CNS and peripheral tissues. Sagittal, 4 μm-thick brain and intestine sections from control fish were treated with SaurPrP1 (1:2000 and 1:250, respectively) and normal endogenous PrP labeling in different anatomical regions was examined. **A**, Optic tectum; **B**, Valvula cerebelli; **C**, Corpus cerebelli; **D**, Nerve fibers in diencephalon; **E**, Neurons in brainstem; **F**, PrP-immunoreactive areas in the intestine. Rectangles indicate areas of magnification shown in the panel directly below. Arrowheads show positively stained regions. SGC, striatum griseum centrale; SPFE, striatum plexiforme et fibrosum extemum; SFM, striatum fibrosum marginale; ML, molecular layer; GL, granular layer, m, plasma membrane; a, axon; c, cytoplasm. se, serosa; mp, myenteric plexus; sp, submucous plexus. Scale bars, 100 μm.
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quantitative) value (Text S1). No lesions were detected in the control fish force-fed normal sheep brain homogenate (Fig. S3 and Table S3B).

Plaque-like deposits were also observed in the brains of the BSE-challenged fish, beginning at earlier timepoints. Initially, at 8 months p.i., the majority of these aggregates were localized in brainstem, less in diencephalon and optic tectum, and even fewer in valvula cerebelli, cerebellum and telencephalon (Figure 3D–F). Just 10% of the deposits were PK-resistant and these had a mean diameter of 5 μm. Subsequently, we observed a general progression in their distribution, size, PK-resistance and morphological features. The incidence of the abnormal deposition was higher in fish sacrificed at earlier time points than at intermediate time points. However, the highest levels were measured at later time points, evocative of the phenomenon described in other prion cross-species transmission studies as an "eclipse" period [30].

Further analysis of the spatial and temporal progression revealed that with increasing time p.i., the deposition became more prominent in rostral brain regions, although caudal regions continued to be affected. By 24 months p.i., deposition in the brains of the BSE-challenged sea bream presented a striking picture, in which three out of five fish showed 500–800 deposits each, 70–85% of which were PK-resistant with a mean diameter of 30 μm. With regard to the remaining two fish, one displayed approximately 150 deposits, 93% of which were PK-resistant and the other showed only limited signs of abnormal aggregation. While deposits continued to be distributed throughout the brain at 24 months p.i., in the three highly affected fish the greatest increases in deposit numbers occurred in brainstem and diencephalon. The progression of the abnormal deposition is apparent in Fig. 3. and summarized in Figs. 4 and 5 and Tables S3A and S4.

In contrast to the BSE-challenged fish, no aggregates were detected at any time in the brains of the control fish fed with normal bovine brain homogenate (Fig. S4 and Table S3A). Notably, none of the brain tissues positive for abnormal deposition showed evidence of neuronal body degeneration. Finally, no residual mammalian PrP^{Sc} was detected using 12F10 and 6H4

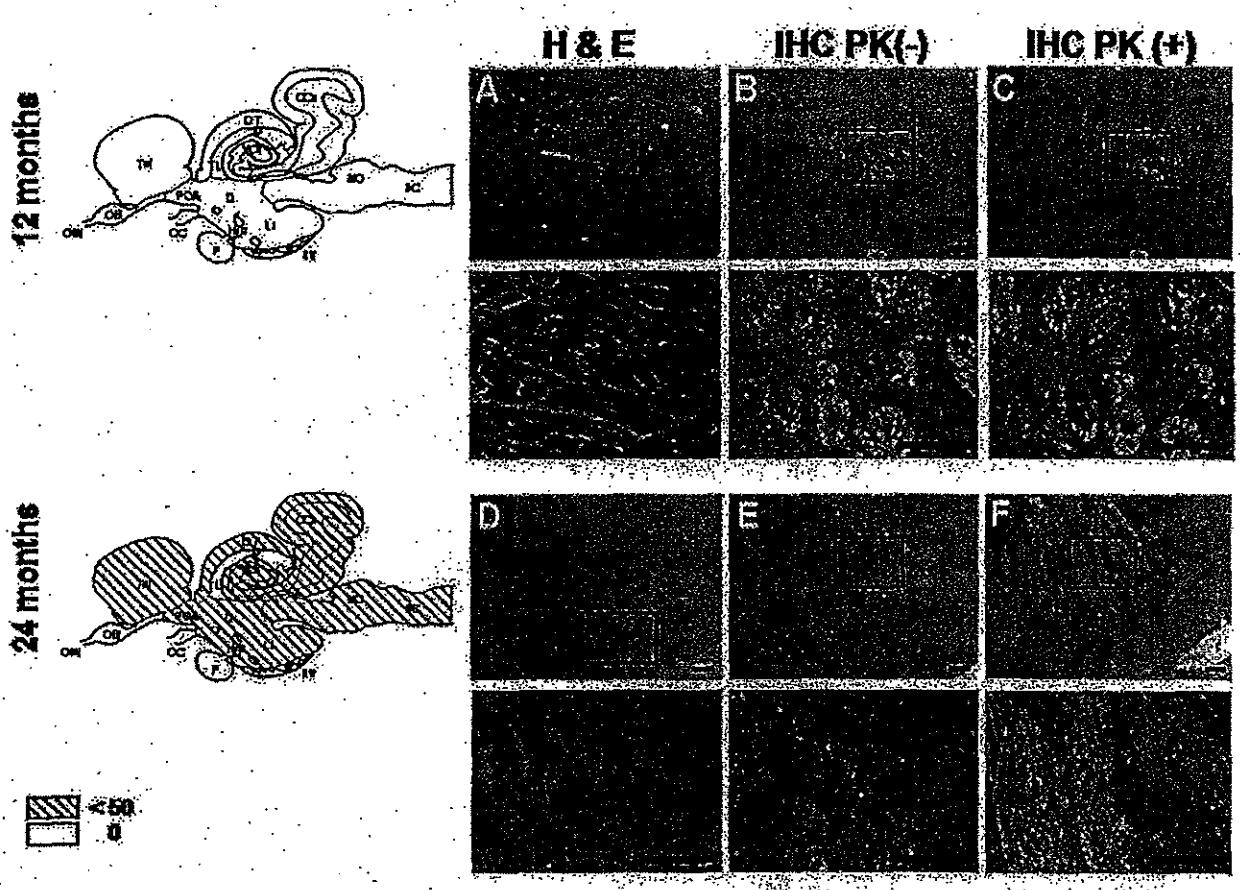

Figure 2. Progression of abnormal deposition in 2 scrapie-challenged fish. Sagittal brain sections from scrapie-challenged fish at 12 and 24 months p.i. were stained with H&E (A, D), or treated with SaurPrP1 (1:2000) without PK-digestion (B, E) and with PK-digestion (C, F). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. CCe, corpus cerebelli; Di, diencephalon; Hyp, hypothalamus; LI, lobi inferioris; MO, medulla oblongata; OB, olfactory bulb; OC, optic chiasm; OIN, olfactory nerve; OT, optic tectum; P, pituitary; POA, preoptic area; SC, spinal cord; Tel, telencephalon; TL, torus longitudinalis; VCe, valvula cerebelli. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Arrowheads indicate the abnormal aggregates. Scale bars, 100 μm.
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monoclonal antibodies (data not shown). Overall, these data suggest that while both TSE strains resulted in similar abnormal brain pathology, the brains of BSE-challenged individuals were more rapidly and severely affected than those of scrapie-challenged fish. BSE, known to be a zoonotic TSE, may represent a thermodynamically favored PrP^{Sc} conformation that is permissive for PrP expressed in a wide range of mammalian species [31]. Despite this permissibility, however, attempts to orally transmit BSE to pigs and chickens have failed [32,33].

To characterize the nature of the deposition, we employed a variety of conventional staining techniques. Congo red-stained deposits in BSE-challenged sea bream brains at 24 months p.i. were congophilic (Figs. 6A and 7B) and birefringent under polarized light (Fig. 6B), suggesting an amyloid or amyloid-like component [34]. No Congo red birefringence was observed in either the control tissues or the scrapie-challenged fish brains (data not shown). While the plaque-like aggregates were prominent with hematoxyline and eosin (H&E) (Fig. 7A), Klüver-Barrera staining for myelin structures and von Kossa staining for calcium deposition both gave negative reactions (data not shown). Deposits were also PAS positive (Fig. 7C) but Alcian blue negative (Fig. 7E), indicating the presence of carbohydrates and the absence of acidic glycosaminoglycans, respectively. Finally, our four anti-fish PrP antisera positively labeled the deposits (see Fig. 7D for SaurPrP1), whereas the 12F10 and 6H4 antibodies did not (data not shown).

Two main types of plaque-like deposits were identified in the brains of the BSE-challenged fish: fibrous, diffusely stained aggregates (Fig. 8A, D, G, J), and those that were more amorphous and dense (Fig. 8B, E, H, K). At 8 months, small aggregates, generally in close proximity to neurofibrils, were detected, whereas the majority of the adjacent fiber bundles remained intact (Fig. 3D–F). At 10 and 12 months the first signs of neurodegeneration appeared as a primitive disorganization of dendrites and axons. By 16 and 18 months, the distention of neurites, mostly in grey matter, was exacerbated. The extensive deconstruction of microfilaments within the axons and the loss of their coherence, especially at 18 months, were detected histopathologically. Aggregates of dystrophic neurites were immunostained with SaurPrP1, exhibiting a diffuse PrP-immunolabeling with some marginal spicule-like projections (data not shown). At 24 months the diffusely immunolabeled aggregates of dystrophic neurites (Fig. 8A, D, G, J) coexisted with deposits that appeared more amorphous, condensed and flocculated and therefore were more intensely stained with all the techniques used (Fig. 8B, E, H, K).

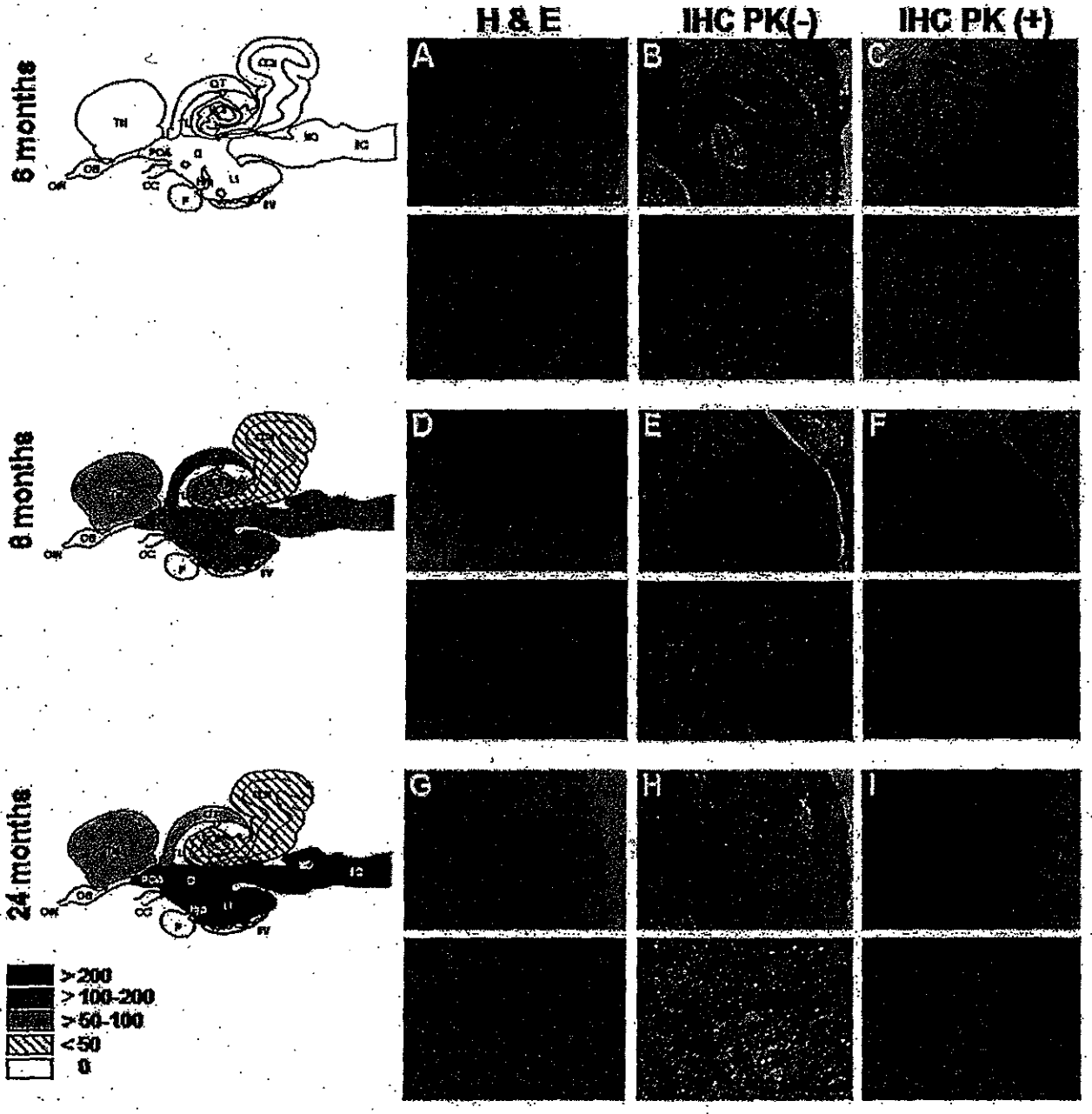

Figure 3. Progression of abnormal deposition in BSE-challenged fish. Sagittal brain sections from BSE-challenged fish taken at the indicated times p.i. were stained with H&E (**A, D, G**), or immunolabeled with SaurPrP1 (1:2000) without PK-digestion (**B, E, H**) and with PK-digestion (**C, F, I**). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. Abbreviations as in Figure 2. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Arrowheads indicate the abnormal aggregates. Scale bars, 100 μm.

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Given the morphological progression of the abnormal deposition with time, it is tempting to hypothesize a scenario, in which the first type of aggregates (Fig. 8A, D, G, J) could have been the developmental ancestor of the second (Fig. 8B, E, H, K) and in which each may illustrate different stages of pathogenesis. The distention of axons and dendrites observed at 10 and 12 months p.i. reflects an initial neurodegenerative process in the brains of the BSE-challenged fish that may have been a very early reaction following exposure to the infectious agent. The complete destruction of the protective outer neurite layers, including the myelin sheath, followed by the disorganization of the microfilaments and microtubuli could have subsequently created the first morphological type of aggregates initially detected at 16 and 18 months. These "pre-mature" deposits mainly consist of dystrophic

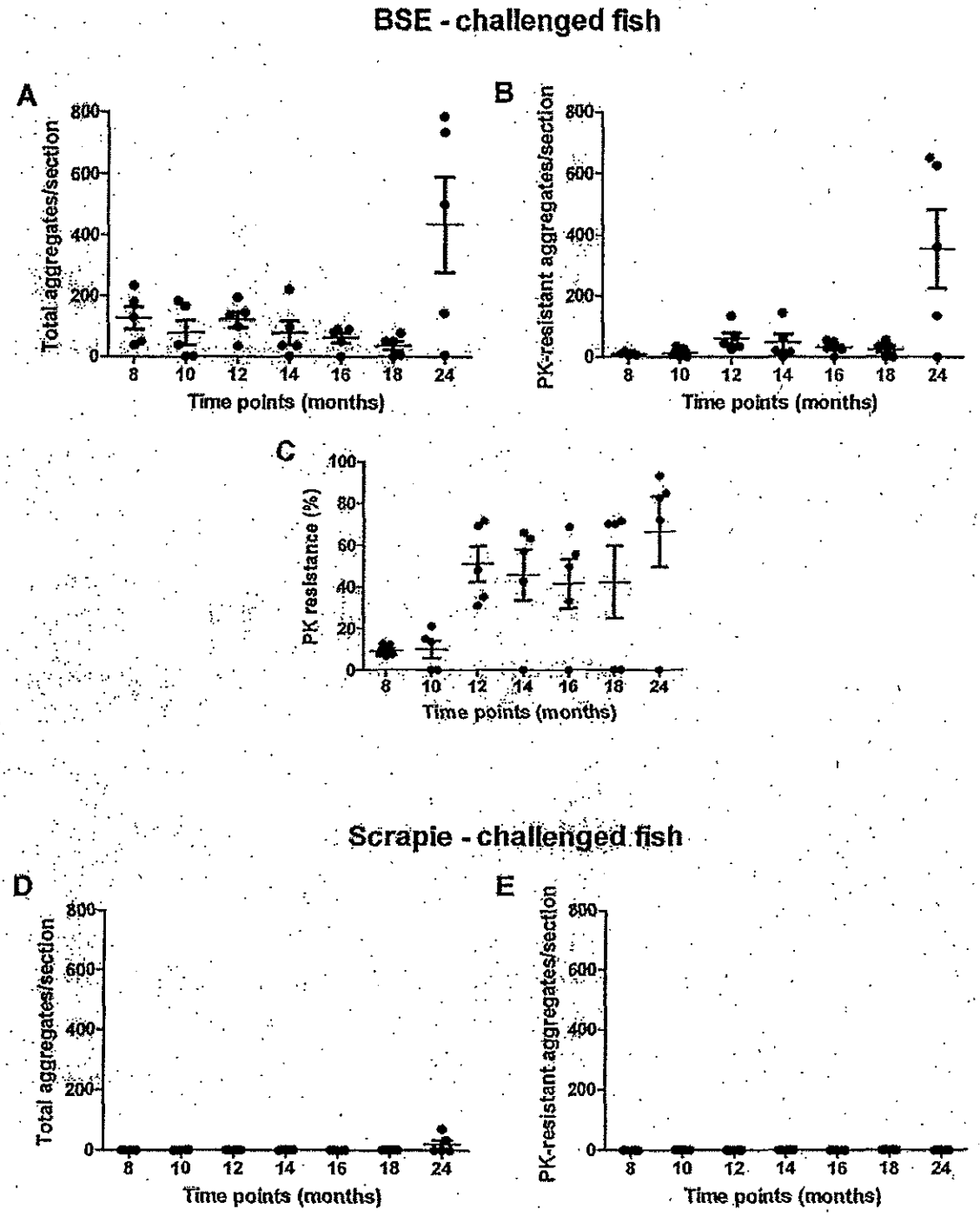

Figure 4. Abnormal deposits in the brains of the BSE- and the scrapie-challenged fish with reference to time. Each dot corresponds to the number of aggregates observed per brain section in each individual before PK-treatment (A, D) and after PK digestion (B, E), or to the percentage of PK-resistant aggregates in each BSE-challenged fish (C). Bars indicate the means and the standard error means (SEMs).
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neurites that have lost their coherence, with spicule-like projections, at their periphery (Fig. 8A, D, G, J). Given that aggregates were partially PK-resistant and showed affinity for Congo red by 24 months, the next step in the progression may have been the complete deconstruction of the fibers leading to the creation of a homogenous, flocculated, extracellular material that we describe as "mature" deposits (Fig. 8B, E, H, K), with increased PrP-immunopositivity, PK-resistance, congophilia and birefringence in polarized light [35]. Fish brains at 24 months post inoculation exhibit both types of abnormal aggregates, including intermediate

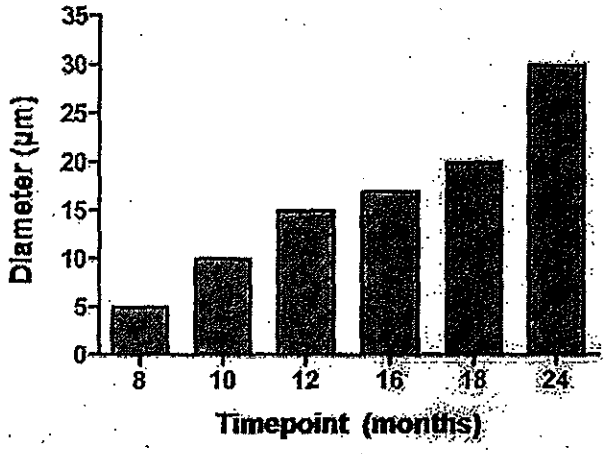

Figure 5. Progressive increase in the size of proteinase K resistant deposits in BSE-challenged fish. The mean diameter of the immunohistochemically detected (Saur*Pr*P1) deposits after proteinase K–digestion is given with reference to time p.i..
doi:10.1371/journal.pone.0006175.g005

states (Fig. 8C, F, I, L) that cannot be easily classified into any of the previously described morphological categories.

The classical neuropathological hallmarks of prion diseases include neurodegeneration, spongiform change and gliosis, while PrP^{Sc} deposition is observed in the majority of TSEs. The lesion profile in the brains of the BSE-challenged fish shares both similarities and differences in comparison to this histological and immunohistochemical pattern of mammalian prion diseases. Specifically, the distribution of abnormal aggregates within the brains of 4 BSE-challenged fish at 24 months p.i. shared certain similarities with the PrP^{Sc} deposition pattern observed in TSE-affected mammalian brain. Notably, the abnormal deposits in sea bream brain were only detected in regions where neuronal parenchyma was present, a feature greatly resembling the location of mammalian prion deposition [36]. Cerebellum was extensively affected, with large fibrous aggregates in the molecular layer and a granule-like deposition profile in the granular layer, a pattern similar to that observed in mammalian TSEs [37]. The abnormal deposition in sea bream brain was also prominent both in the lateral nucleus of the ventral telencephalic area, a fish counterpart to the basal nucleus of Meynert in mammals [38], and the lateral telencephalic pallium, homologue to the mammalian hippocampus [22]. Both thalamus and diencephalon displayed numerous aggregates, most of which were interspersed within neuronal fibers. In striking contrast to the general neuropathological profile of mammalian TSEs, however, no vacuoles were observed in any regions of the fish brains examined. While spongiosis is a main characteristic in most prion diseases, it must be noted that in certain TSE subtypes there is little or no spongiform change. Such has been the case in patients suffering from FFI, an inherited human prion disease [39].

Evidence of neurodegeneration, although distinct from that commonly associated with mammalian prion disease, was apparent in many brain regions, primarily in places where the abnormal deposition was located adjacent to or within complexes of neurites (Fig. 8C, L). It is important to note, however, that no degeneration associated with neuronal somata was detected in any of the anatomical regions examined. The absence of classically defined neurodegeneration might be related to the ability of fish to produce new neurons continuously throughout their lifetime. It is known, for instance, that adult fish can regenerate damaged retinal tissue, optic axons and descending brainstem axons, leading to functional recovery [40,41]. In fact, this ability of adult fish for CNS regeneration has been postulated to explain the asymptomatic carrier state of halibut persistently infected with nodavirus

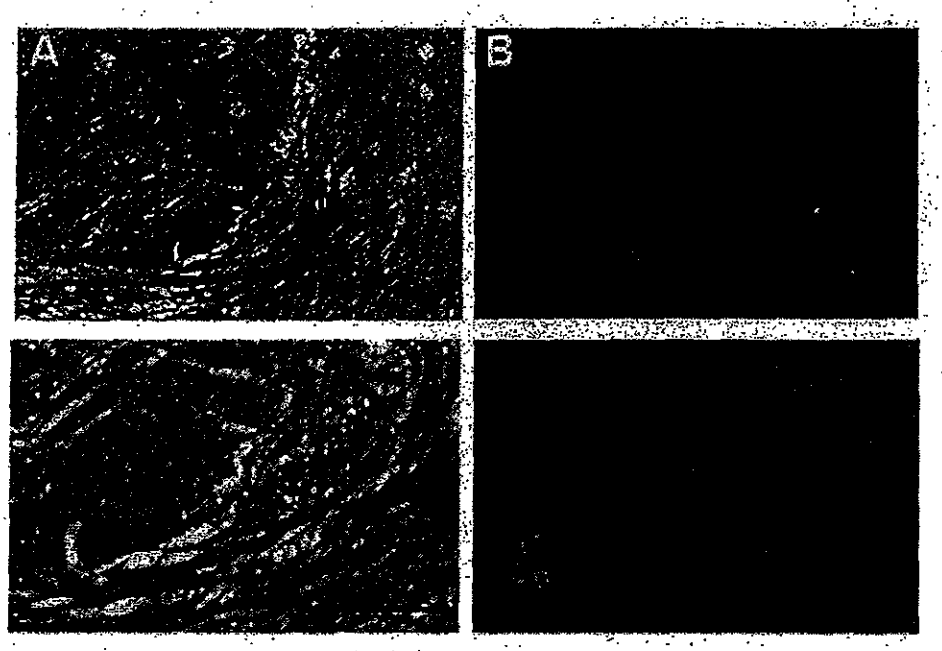

Figure 6. Congo red staining of deposits in the brain of BSE-challenged sea bream. A sagittal, 10 µm-brain section from a BSE-challenged individual, 24 months p.i., was stained with Congo red. **A**, Diencephalon with light microscopy; **B**, Same region in polarized light. Rectangles indicate areas of magnification shown in the panel directly below. Arrowheads indicate the abnormal aggregates. Scale bars, 100 µm.
doi:10.1371/journal.pone.0006175.g006

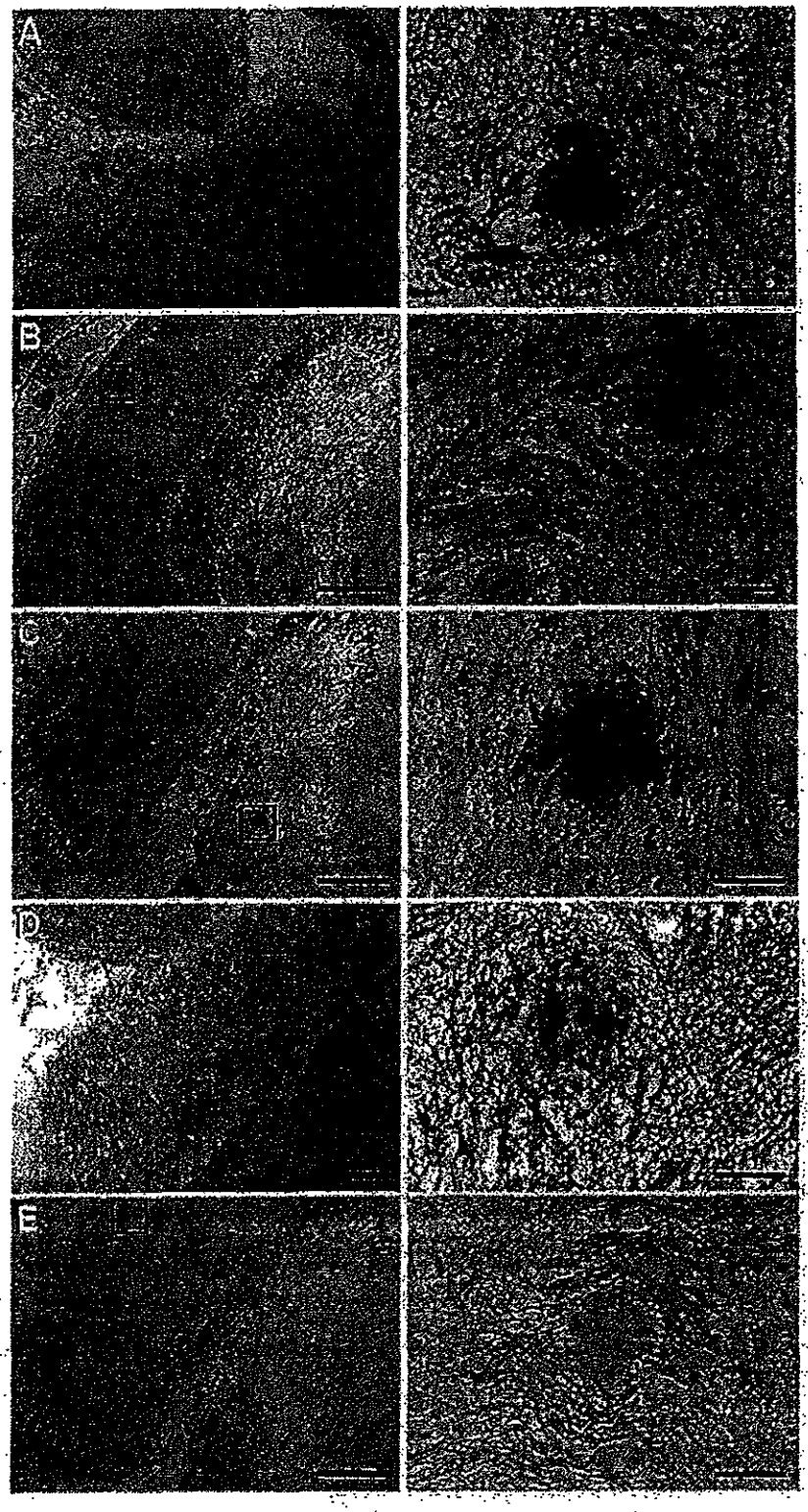

Figure 7. Staining of an aggregate in the brain of a BSE-challenged fish 24 months p.i. with different techniques. **A**, H&E; **B**, Congo red in normal light; **C**, PAS; **D**, IHC (SaurPrP1) after PK-digestion; **E**, Alcian blue. Rectangles in the left panel indicate areas of magnification shown in the right panel. Scale bars, 100 μm (left panel) or 10 μm (right panel).
doi:10.1371/journal.pone.0006175.g007

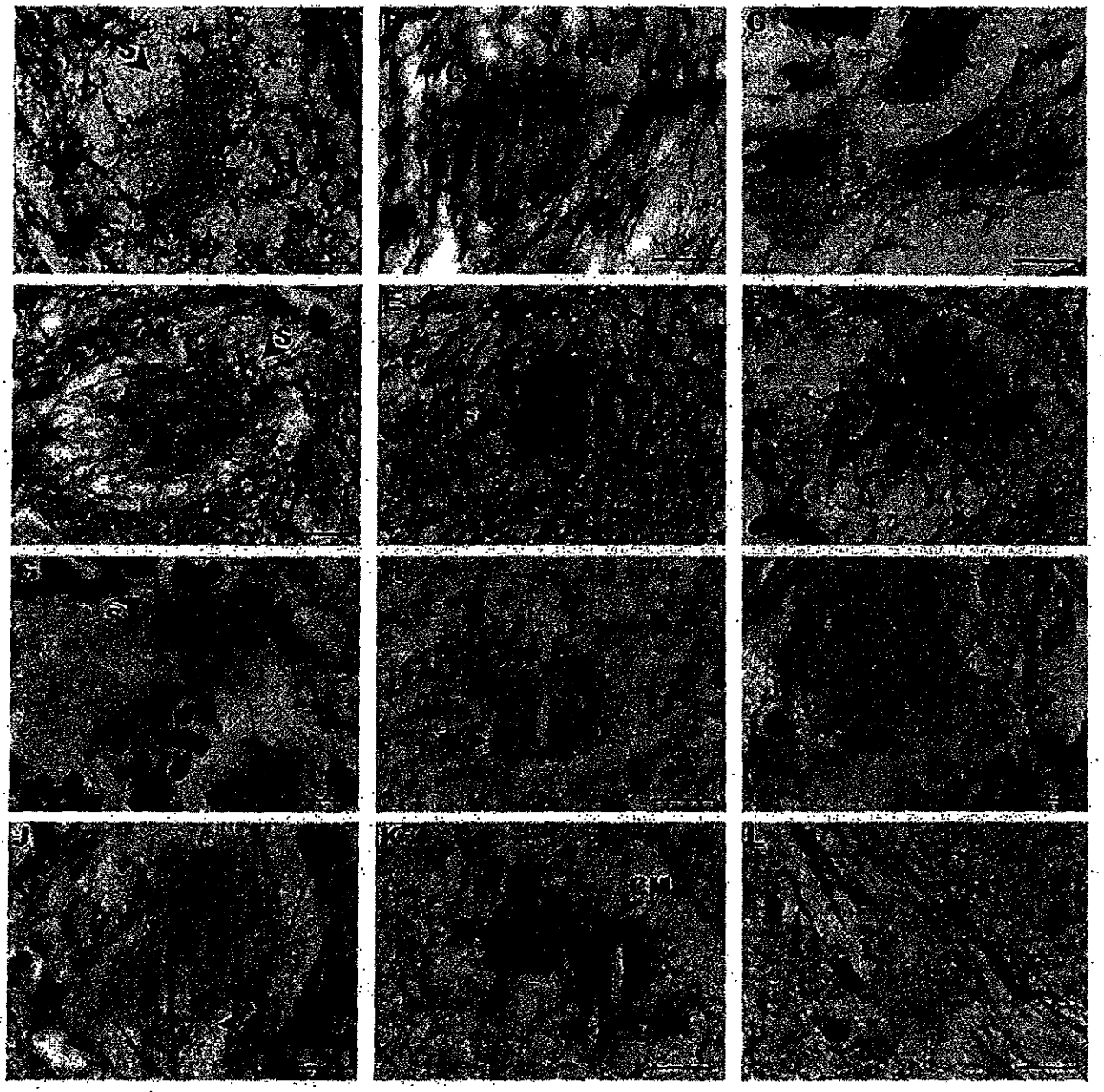

Figure 8. Morphology of the abnormal deposition in the brains of the BSE-challenged fish 24 months p.i.. Sagittal 4 µm-thick brain sections where stained with hematoxylin without a counterstain. pH variation renders the deposits visible by light microscopy. The panels on the left show diffuse "pre-mature" deposits in **A,** diencephalon; **D,** optic tectum; **G,** cerebellum; **J,** brainstem. The middle panels display examples of compact "mature" deposition in **B,** diencephalon; **E,** optic tectum; **H,** cerebellum; **K,** telencephalon. The panels at the right show intermediate stage-abnormal deposition within fiber bundles that disrupts synapses in **C,** diencephalon; **F,** optic tectum; **I,** valvula cerebelli; **L,** telencephalon. S, spicule-like projections; CM, condensed material. Scale bars, 10 µm.
doi:10.1371/journal.pone.0006175.g008

[42]. In the present study it may have contributed to the "eclipse-like" temporal appearance of the abnormal deposition, as well as to the lack of clinical symptoms in our BSE-challenged fish.

Despite the positive IHC results, western blotting failed to detect PK-resistant PrP isoforms in the TSE-challenged fish brains (Fig. S5), possibly because the whole brain homogenates used did not have a high enough concentration of PK-resistant PrP to allow detection. In fact, it is clear from the IHC results that even at 24 months p.i., the brain regions associated with the abnormal deposits constitute only a small percentage of the whole brain mass.

The results of this TSE transmission study with gilthead sea bream indicate the development of a CNS histopathology in the brains of the fish challenged with the TSE-inocula. This neuropathology displays characteristics resembling a novel fish amyloidosis more than a classical TSE. Specifically, while the fish

in our study showed no brain spongiosis and no clinical abnormalities, we did find numerous plaque-like deposits in the brains of a significant proportion of the BSE-challenged fish, especially. Although much of the PrP associated with these deposits is PK-sensitive, this should not be taken as an indicator of low potential infectivity, as instances of clinical prion disease, and even infectivity, associated with extremely low levels of detectable PK-resistant PrP have been reported [43–45].

In light of the serious ramifications that would follow an unequivocal demonstration of prion disease transmission to fish, it must be emphasized here that the abnormal deposition we observed in the brains of the TSE-challenged fish could possibly have resulted from pathogenic factors other than the prions they were fed. Despite the fact that no such naturally occurring, cross-species infections from mammals to fish have ever been reported [46], we cannot completely rule out this possibility. Thus, however unlikely, one must consider the possibility that the brains used to prepare the inocula for the TSE challenge were infected with an undetected virus or bacteria in addition to the scrapie or BSE present. Together, the time course of brain lesion appearance, i.e. months not days, the ability of the agent to survive the oral challenge route, the absence of brain histopathology in any of the control groups and the production of novel histological lesions in both the BSE- and the scrapie-challenged fish, in the absence of inflammation, however, make this possibility a remote one. A more plausible alternate explanation would be that the amyloidogenic nature of the TSE-inocula might have contributed to the development of a novel fish brain amyloidosis.

Infectivity and transmissibility are crucial issues that still need to be addressed. From a public health standpoint, the transmissibility of each prion strain and the relative ease with which it crosses species barriers, are its most significant characteristics. The spectrum of prionopathies, which has broadened in recent years, includes prion diseases that are not readily transmissible (*e.g.* some GSS cases), prion strains often associated with negligible clinical symptoms (*e.g.* the Nor98 scrapie strain), and even some without detectable PrP^{Sc} (*e.g.* PSPr) [44,47,48]. It is clear, then, that the evaluation and identification of both unusual prion diseases and prion diseases affecting unusual hosts is a complex task, requiring lengthy studies of pathogenesis, infectivity and transmissibility [49]. Until ongoing transmission studies using "bovinized" transgenic mice are completed, the possibility that the affected sea bream brain tissue might be infectious, must be taken seriously in any consideration to lift EU feed bans, especially those related to farmed fish.

Materials and Methods

Ethics Statement

All fish used in the experiments described in this work were treated in accordance with EU Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes.

Fish

Sixteen hundred gilthead sea bream of approximately 20 g weight were purchased from a commercial farm (Interfish, Greece). At the commercial farm, before purchase, fish were fed commercial pellets (Biomar), none of which contained protein sources derived from land animal products. After transportation to the laboratory (Fisheries Research Institute, Kavala, Greece), they were maintained at 18°C in temperature-controlled recirculating water tanks. After a two week adaptation period, the fish were divided into groups of 200 in separate tanks. The fish were allowed a further three weeks of acclimatization before experimental manipulations were initiated.

Preparation of inocula

For the force feeding of the fish, 10% (w/v) brain homogenates from scrapie-infected sheep, healthy control sheep, BSE-infected cow and healthy control cow were prepared in PBS (pH 7.4). For the sheep brain homogenates both cerebellum and brainstem from two animals were used (kindly provided by Dr. P. Toumazos, Veterinary Services, Cypriot Ministry of Agriculture), while the bovine brain homogenates were each prepared from the brainstem of a single animal. The BSE sample (RBSE 21028), taken in 1991 from a female Fresian two months after disease onset, was kindly provided by Dr. Ian Dexter, Pathology Department, Veterinary Laboratories Agency, Weybridge, UK. As healthy herdmate tissue was not available, the healthy control bovine brainstem was taken from a local Greek cow in 2002. All brain samples were stored at −80°C prior to use.

Challenge and maintenance

For inoculations, fish were removed from the tanks and mildly anaesthetized with 0.3% ethylene glycol monophenyl ether. Following anaesthesia, each fish was force-fed 100 µl brain homogenate. In total, 2 groups of 400 fish each, were each treated with scrapie-infected or control sheep brain homogenate and 2 groups of 200 fish each were each treated with BSE-infected or control bovine brain homogenate. For both the experimental and control groups, the force-feeding procedure was repeated fortnightly for a total of five treatments, so that the cumulative inoculum for each fish was 50 mg brain equivalents. Following the inoculation period all fish were kept on a maintenance diet with commercially available chow to prevent excessive growth and overcrowding during the multiyear study period. Data regarding maintenance of the fish, mortality due to technical and natural causes and sampling are shown in Table S2.

Clinical examination

Clinical evaluation of the fish in each tank was made on a daily basis, checking especially for any behavioral or swimming abnormalities.

Histopathological evaluation

Individuals from each group (5 TSE-treated and 5 controls) were sacrificed at regular selected time points post inoculation (3, 6, 8, 10, 12, 14, 16, 18, 24 months) and tissue samples, including brain, spleen and intestine, were taken. Tissues were fixed in buffered formalin (pH 7.4), embedded in paraffin wax and finally 4 µm-thick serial sections were subjected to conventional staining with a variety of staining techniques including H&E, PAS, Alcian blue, von Kossa and Klüver-Barrera. The resulting sections were examined histologically using light microscopy (Axioplan 2 Imaging System, Zeiss). Tissue pictures were taken using the Nikon Digital Sight DS-SMc visualizing system.

Congo red staining

For the identification of possible amyloid-like structures, 10 µm-thick brain sections were deparaffinized, stained with 0.5% Congo red (Merck, Darmstadt, Germany) alcohol solution for 15 minutes, destained in 0.2% KOH, subsequently counterstained with Mayer's hematoxyline, and after a short dehydration, they were finally cleared in xylene. The stained sections were observed microscopically under both normal and polarized light (Axiolab

Carl Zeiss, rotatable analyzer +/−5°, 6×25, rotatable compensator Lambda, +/−5°, 6×25).

Generation of polyclonal antisera

The presumed mature sequences spanning residues 24–580 of zebrafish (*Danio rerio*) PrP-1 and residues 18–539 of zebrafish PrP-2 (sequence data provided by Dr. Edward Málaga-Trillo, Department of Biology, University of Konstanz), were each amplified from genomic DNA, whereas the mature sequence of gilthead sea bream (*Sparus aurata*) PrP-1 spanning residues 26–475 was cloned from plasmid DNA. All three were cloned into the pET21d DNA vector (Novagen, San Diego, CA) to produce recombinant proteins tagged with six histidine residues at the C-terminus. After sequence verification by double-stranded sequencing, the recombinant proteins were expressed in BL21 (DE3) *E.coli* (Stratagene, La Jolla, CA) with IPTG induction from single clone colonies. The recombinant proteins were purified under denaturing conditions from cell lysates on Ni-NTA agarose columns (Qiagen, Hilden, Germany) and then specifically eluted with imidazole. The polyclonal antisera ZebPrP1, ZebPrP2 and SaurPrP1 were raised against the purified zebrafish PrP-1, zebrafish PrP-2 and sea bream PrP-1 proteins respectively, by 3 successive subcutaneous inoculations of rabbits with 150 to 200 µg of recombinant protein at 4 weeks intervals. All pertinent sequence data are deposited in GenBank and the accession numbers are given at the end of the manuscript.

Affinity purification of SaurPrP1 polyclonal antiserum

2 aliquots containing 150 µg of gilthead sea bream recombinant PrP-1 protein each, were loaded onto 10% polyacrylamide gels and after SDS-PAGE, they were each electrotransferred to a nitrocellulose or a PVDF membrane. After electrophoresis the two membranes were stained with amido black staining solution (0.1%) and the protein-containing membrane pieces were finally excised. After a blocking step in 50 mM Tris HCl [pH 7.4], 150 mM NaCl, 0.05% Tween 20 (TBST) containing 5% milk, for 1 hr at RT, each membrane piece was probed with blocking buffer containing 500 µl of SaurPrP1 antiserum, at 4°C overnight. Following several washes, the IgGs that specifically bound to PrP-1, were finally eluted from the membranes with 0.2 M Glycine.HCl [pH 2.5], for 5 min at 4°C. Each eluate was neutralized with 2 M Tris HCl [pH 9.0], and then dialyzed overnight at 4°C in 50 mM Tris HCl [pH 7.4], 150 mM NaCl (TBS), using 100 mm-Spectra/Por molecularporous membrane tubing (Spectrum Medical Industries, Los Angeles, USA). Following dialysis, the purified IgGs were saturated in buffer containing 20 mM Tris HCl [pH 8.4], 150 mM NaCl, 5 mM EDTA, 1% gelatin, 0.1% BSA.

Depletion of recombinant gilthead sea bream PrP-1 – specific immunoglobulin fraction from SaurPrP1 polyclonal antiserum

SaurPrP1 polyclonal antibody was diluted in phosphate buffered saline (1:2000), containing 5% normal goat serum, 2.5% BSA and 0.05% Tween 20. The antibody was incubated with 0.6 mM of recombinant gilthead sea bream PrP-1 protein at 4°C overnight. The depleted antiserum was briefly centrifuged before use in all negative immunohistochemistry control experiments.

Immunohistochemistry

Four different polyclonal anti-PrP antibodies were used for the immunohistochemical detection of the endogenous PrP proteins of sea bream, namely ZebPrP1 (1:1000), ZebPrP2 (1:1000), SaurPrP1 (1:2000) and FuguPrP1 (1:500), the latter being raised by our group against PrP-1 protein of *Takifugu rubripes*. The commercially available monoclonal antibody, 12F10 (Cayman Chemical, Ann Arbor, MI), raised against amino acids 142–160 of human PrP, was used for the detection of residual mammalian PrP (1:200), since it also displays cross-reactivity with both ovine and bovine PrP. All paraffin sections were cut at 4 µm thickness. Depending on the prion protein of interest, PrP^{C} or PrP^{Sc}, two different pretreatment protocols were used. For PrP^{C} labeling, an antigen retrieval step was performed by boiling in citrate-buffered saline [pH 6.0] for 7 minutes before the staining procedure. For PrP^{Sc} detection, the sections were hydrated-autoclaved at 121°C for 30 minutes, then incubated for 5 minutes in 90% formic acid prior to an 8 minutes-incubation with proteinase K (Dako, Glostrup, Denmark) at RT. Sections were treated with appropriate biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA) and visualized using the avidin-biotin method-based Vectastain Elite ABC and the Diaminobezidine substrate kits (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Negative controls for immunohistochemistry involved omitting the primary antibody. Staining with polyclonal (anti 14-3-3β, Santa Cruz, California, USA) and monoclonal antibodies (12F10, 6H4) raised against proteins of mammalian origin was also performed. The mouse anti-tubulin monoclonal antibody (Abcam, Cambridge, UK) and the monoclonal SAF84 antibody (raised against SAF preparation from infected hamster brain, assumed epitope 126–164) were used as well.

PrP^{Sc} enrichment and Western blot analysis

Western blot analysis of potentially enriched mammalian and teleost PrP^{Sc} was performed on brain homogenates from BSE-infected cows, scrapie-infected sheep, and TSE-challenged fish. Briefly, aliquots of 10% (w/v) brain homogenate were digested for 1 hr at 37°C with proteinase K at 25 µg/ml for sheep, 30 µg/ml for cow and 0.1–10 µg/ml for sea bream. PMSF (5 mM) was added to stop the protease digestion and PrP^{Sc} was precipitated with NaCl (10%) (w/v). The pellet was washed with 25 mM Tris HCl [pH 8.8] containing 0.05% sarkosyl and then resuspended in an appropriate volume of 2.5× O'Farrell buffer for gel electrophoresis.

For western blot analysis, untreated and proteinase K treated brain homogenates were analyzed by SDS-PAGE on 12% polyacrylamide gels and the separated proteins were then transferred onto PVDF membranes. After blocking with phosphate buffered saline containing 0.1% Tween 20 (PBST) and 5% milk, the immunoblots were probed with the fish-PrP specific polyclonal antisera, ZebPrP1 (1:10000), ZebPrP2 (1:35000), SaurPrP1 (1:20000), FuguPrP1 (1:20000), and the monoclonal antibody 6H4 (1:5000) (Prionics, Zurich, Switzerland) overnight at 4°C. After washing, they were incubated for 1 hr with either alkaline-phosphatase or horseradish-peroxidase conjugated secondary goat anti-rabbit or anti-mouse antibodies (Pierce, Rockford, IL) diluted 1:10000 in PBST. The blots were developed using the CDP-Star chemiluminescent substrate (NE Biolabs, Beverly, MA), or the ECL Western blotting Substrate (Pierce, Rockford, IL), depending on the secondary antibody and according to the manufacturer's instructions.

GenBank Accession Numbers

Danio rerio prion protein 1 coding sequuence: AY438683
Danio rerio prion protein 1: AAS00159
Danio rerio prion protein 2 coding sequence: AY438684

Danio rerio prion protein 2: AAS00160
Sparus aurata prion protein 1 coding sequence: ABB90540

Supporting Information

Table S1 Percentage amino acid sequence homology between prion proteins of different species. Full sequences were aligned. The NCBI accession numbers of sequence data are: Homo sapien (human), AAC78725; Bos Taurus (cow), AAD19998; Ovis aries (sheep), CAE00188; Mus musculus (mouse), AAH06703; Mesocricetus auratus (hamster), AAA37092; Takifugu rubripes (fugu), AAN38988; Danio rerio (zebrafish) prion protein 1, AAS00159; Danio rerio prion protein 2, AAS00160; Sparus aurata (gilthead sea bream) prion protein 1, ABB90540. Sequence alignments were performed by ALIGN (version 2, Myers and Miller, CABIOS (1989) 4:11–17).
Found at: doi:10.1371/journal.pone.0006175.s001 (0.09 MB TIF)

Table S2 Cumulative record of the number of fish maintained post challenge. A, Fish inoculated with either scrapie- or normal ovine brain homogenate. B, Fish inoculated with either BSE- or normal bovine brain homogenate.
Found at: doi:10.1371/journal.pone.0006175.s002 (0.04 MB DOC)

Table S3 Cumulative record of brain tissue samples examined. A, BSE-challenged and bovine control fish samples. B, Srapie-challenged and ovine control fish samples.
Found at: doi:10.1371/journal.pone.0006175.s003 (0.14 MB DOC)

Table S4 Cytoanatomical analysis of brains from BSE-challenged fish sacrificed 24 months post challenge. The deposits have been classified into 2 morphological categories. F, fibrillary, >10 μm in diameter; NF, non fibrillary, circular<10 μm in diameter. Plus and minus symbols indicate the abundance of deposits: −, 0; +, 1–5; ++, 6–15; +++, 16–50, ++++, >50. NP, anatomical region not present in section; Mol L, molecular layer; Gran L, granular layer; WM, white matter; Cx, cortex; Ce, cerebellum; Vc, valvula cerebelli; Tel, telencephalon; Di, diencephalon; OT, optic tectum; Br. st., brain stem.
Found at: doi:10.1371/journal.pone.0006175.s004 (0.13 MB TIF)

Figure S1 Comparison of antibody specificities for the PrPs of gilthead sea bream, cow and sheep by western blot analysis. A, Five 0.4 mg brain equivalent-aliquots of gilthead sea bream brain homogenate were loaded onto a 12% SDS-PAGE gel. B, Alternating lanes of a 12% SDS-PAGE gel were loaded with 3 mg tissue equivalents of PrPSc-enriched (see Materials and Methods) bovine BSE brain homogenate (lanes 1, 3, 5, 7 & 9) and ovine scrapie brain homogenate (lanes 2, 4, 6, 8 & 10). The electrophoretically separated proteins were transferred to PVDF membranes that were cut into four sections (in B, each section included two adjacent lanes). Each section was stained with one of five primary antibodies: 6H4 (1:5000; lanes A1, B1, B2); FuguPrP1 (1:20000; lanes A2, B3, B4); ZebPrP1 (1:10000; lanes A3, B5, B6); ZebPrP2 (1:35000; lanes A4, B7, B8); SaurPrP1 (1:20000; lanes A5, B9, B10). After incubation with the appropriate alkaline-phosphatase-conjugated secondary antibody, the blots were developed with the CDP-Star reagent. The arrow heads indicate the positions of the molecular mass markers: A, 62 kDa and 47.5 kDa; B, 32.5 kDa.
Found at: doi:10.1371/journal.pone.0006175.s005 (0.28 MB TIF)

Figure S2 Antibody specificity in IHC. Sagittal, 4 μm-thick serial brain sections from control gilthead sea bream were treated immunohistochemically with four different primary antibodies, without proteinase K digestion. A, SaurPrP1 (1:2000); B, ZebPrP2 (1:2000); C, Pre-immune serum from the rabbit in which SaurPrP1 was raised (1:2000); D, PrP-specific immunoglobulin-depleted SaurPrP1 (1:2000). Arrowheads indicate the existence (A, B) or absence (C, D) of PrP-immunopositivity. SGP, striatum griseum periventriculare; SFP, striatum fibrosum profundum; SGC, striatum griseum centrale; SPFE, striatum plexiforme et fibrosum externum; SFM, striatum fibrosum marginale; ML, molecular layer of the valvula cerebelli; GL, granular layer of the valvula cerebelli. Scale bars, 100 μm.
Found at: doi:10.1371/journal.pone.0006175.s006 (8.23 MB TIF)

Figure S3 Temporal observation of the brains from control fish challenged with normal ovine brain homogenate. Sagittal brain sections taken at 12 and 24 months p.i. from fish challenged with normal ovine brain homogenate were stained with H&E (A, D), or immunolabeled with SaurPrP1 (1:2000) without PK-digestion (B, E) and with PK-digestion (C, F). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. Abbreviations as in Figure 2 of the main manuscript. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Scale bars, 100 μm.
Found at: doi:10.1371/journal.pone.0006175.s007 (3.00 MB TIF)

Figure S4 Temporal observation of the brains from control fish challenged with normal bovine brain homogenate. Sagittal brain sections taken at the indicated timepoints p.i. from fish challenged with normal bovine brain homogenate were stained with H&E (A, D, G), or immunolabeled with SaurPrP1 (1:2000) without PK-digestion (B, E, H) and with PK-digestion (C, F, I). Images show diencephalon. The mean number of deposits (per section of fish containing deposits) observed in different brain regions without PK-treatment is indicated by the fill-type in the schematic drawings at the far left. Abbreviations as in Figure 2 of the main manuscript. The following areas were not examined: OB; OIN; OC; P. Rectangles indicate areas of magnification shown in the panels directly below. Scale bars, 100 μm.
Found at: doi:10.1371/journal.pone.0006175.s008 (4.48 MB TIF)

Figure S5 Sensitivity to proteinase K treatment of TSE-challenged sea bream brain tissues 24 months p.i.. After a short purification treatment, 0.4 mg brain equivalents from brain homogenates of either scrapie- (lanes 1, 3, 5 & 7) or BSE-challenged (lanes 2, 4, 6 & 8) fish, were digested with increasing proteinase K concentrations (0 μg/ml, lanes 1 & 2; 0.1 μg/ml, lanes 3 & 4; 1 μg/ml, lanes 5 & 6; 10 μg/ml, lanes 7 & 8) for 1 hr at 37°C. The samples were analyzed on a 12% SDS-PAGE gel, then electrotransferred onto a PVDF membrane and probed with SaurPrP1 polyclonal antibody (1:20000). After incubation with the appropriate secondary antibody, the immunoblots were finally developed with the ECL western blotting substrate. Arrowhead, 47.5 kDa.
Found at: doi:10.1371/journal.pone.0006175.s009 (0.27 MB TIF)

Text S1 Statistical analysis of data derived from the scrapie - challenged group.
Found at: doi:10.1371/journal.pone.0006175.s010 (0.02 MB DOC)

Acknowledgments

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This article is dedicated to Giorgos Arvanitidis, champion of the Olympus Marathon.

Author Contributions

Conceived and designed the experiments: ES CP EK GK TS. Performed the experiments: ES CP KT SP EE FA. Analyzed the data: ES CP NG AN EK GK TS. Contributed reagents/materials/analysis tools: ES CP KT SP EE FA NG AN EK GK TS. Wrote the paper: ES CP TS.

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医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
			2009年10月26日	該当なし	

一般的名称	人 C1-インアクチベーター	研究報告の公表状況	Report to the Board of Directors: Current Status of TSEs and Transfusion Safety :AABB Weekly Report 2009年10月22日号Vol.15 No.39	公表国 米国
販売名（企業名）	①ベリナートP ②ベリナートP 静注用 500 （CSL ベーリング株式会社）			

研究報告の概要

問題点（プリオン遺伝子のコドン 129 遺伝子が MM 型と同一でない特定の人達の中から見つかった）

TTD（Transfusion Transmitted Diseases）Committee が伝達性海綿状脳症（TSE）の状況および輸血の安全性に関する報告書を AABB Board の次回会合で提出予定である。報告の概要は以下のとおりである。

数人の感染者（症状発現なし）が、プリオン遺伝子のコドン 129 遺伝子が MM 型と同一でない特定の人達の中から見つかり、疾患の第二波の可能性や潜在的なキャリアのグループが拡大することに対して懸念されている。供血後に vCJD を発症した供血者 3 人から採取された血液の輸血により vCJD プリオンが合計 4 人に伝播している。

受血者の 3 例は vCJD を発症したが、1 例は vCJD の症状がなく、他の原因で死亡した。その患者の脾臓とリンパ節で異常プリオン蛋白が発見されたが、興味あることにその患者のプリオン遺伝子のコドン 129 が MV 型であることが判明した。非 MM 遺伝子型での感染の潜在的な重要性についての検討を以下に簡潔に示す。最近、供血後に vCJD を発症した供血者の血漿が含まれていたことが判明した血液凝固第 VIII 因子製剤を投与された一人の血友病患者において病原性 vCJD プリオンが発見された。その患者は vCJD の症状が現れなかった。モデリング研究では感染はおそらく血漿分画製剤によると示唆された。アメリカ赤十字社は、Transfusion に掲載された論文で、供血後に古典的 CJD を発症した供血者から伝播するエビデンスが不足していると報告している。その研究では、供血後に CJD を発症した 36 人の供血者の血液を投与された 436 人の受血者のルックバック調査をしたが、CJD の症例はなかった。暴露経験のある受血者を英国の vCJD に暴露した患者のコホートスタディーと比較すると CJD を発症するリスクが有意に低かった（p = 0.012）。この研究は、輸血による古典的 CJD の感染性に関するエビデンスが不足していることをサポートしている。血友病患者及び非 MM 型の特定の人達から vCJD プリオンが発見された結果、米国 FDA は米国由来の血漿分画製剤の受血者に関する vCJD 伝播のリスクモデルを改訂した。新しいモデルは 2009 年 6 月に開催された伝達性海綿状脳症諮問委員会で発表された。一人当たりの年間の最低の推定リスクは 5 倍から 18 倍に上昇したが、まだ 1200 万人に 1 人の低さであり、最大の推定リスクは 12000 人に 1 人のまま変わらず、FDA は、米国患者へのリスクは「極めて低い」と見なしている。

ヒトプリオン遺伝子のコドン 129 には数種のバリエーションがあり、3 つの主要な遺伝子型、MM 型、MV 型と VV 型がある。

現在まで評価された全ての症候性の vCJD 患者のコドン 129 の遺伝子型が MM 型であった。

英国人の約 40%がこの遺伝子型である。しかし病原性プリオンが MV または VV 遺伝子型である無症候性の人達で発見されている。これにより 2 つの疑問が生じてくる。最初の疑問は、非 MM 遺伝子型の人達での vCJD の「第二波」が起こるかどうかということである。これはたぶん潜伏期間がより長期化したためである。二番目は非 MM 遺伝子型を持つ人達が vCJD プリオンの感染キャリアになり得るかということである。2 つとも解決が待たれる。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
当社製品を製造する原料血漿は、ドイツ、米国、オーストリア由来であり、また英国等の滞在期間、通算滞在歴に基づき供血停止基準を設けて収集している。 本剤の添付文書に、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、本剤の投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与することを記載し、注意喚起している。	今後とも新しい感染症に関する情報収集に努める所存である。

ABJ20092206

Report to the Board of Directors: Current Status of TSEs and Transfusion Safety

Executive Summary:

There continues to be concern about transmission of variant Creutzfeldt Jakob disease (vCJD) by blood transfusion, with a total of 4 reported cases, plus a potential case of transmission of the prion via a UK-derived plasma derivative, known to have contained potentially infectious donations. Some infections (but no disease) have been found among individuals who are not homozygous for MM at the 129 codon of the PRP gene, raising concern about a possible second wave of disease and an expanded group of potential carriers. There has been no published progress in blood donor testing technologies. One method for prion removal from red cell concentrates has been evaluated by authorities in the UK and Ireland.

Information about transfusion transmitted infectivity:

There have now been a total of four cases of transmission of the vCJD prion by transfusion of blood collected from three donors who subsequently developed vCJD. Three of the cases resulted in the development of vCJD in the recipient, while one was detected in the spleen and one lymph node of a transfused patient who died of other causes. This individual had no symptoms of vCJD (1, 2). Interestingly, he was found to be (MV) heterozygous at codon 129 of the PrP gene. The potential significance of infection among non MM genotypes is discussed briefly below. More recently, evidence of pathologic vCJD prions was found in a hemophilic recipient of F VIII concentrates known to have included plasma from a donor who subsequently developed vCJD (3). Again, the recipient patient was free of vCJD symptoms. Modeling studies imply that the infection was most likely to have come from the plasma products (4).

In a paper published in Transfusion, American Red Cross authors reported on the absence of evidence of transmission of classic CJD from donors who subsequently developed the disease. The study involved lookback on 436 recipients of donations from a total of 36 donors who developed the disease subsequent to their donation, finding no cases of CJD among the recipients. A subset of recipients with exposure histories comparable to the UK cohort of patients exposed to vCJD was shown to be at lower risk of developing CJD: a statistically significant ($p = 0.012$) observation. This supports the absence of infectivity of classic CJD via transfusion (5).

Risk modeling for recipients of plasma derivatives in the US:

As a result of the finding of the vCJD prion in a hemophilia patient (described above), and the findings of vCJD prions among non MM individuals, the US FDA has revised its model of the vCJD transmission risk for recipients of US-derived plasma derivatives. The new models were presented at a meeting of the Transmissible Spongiform Encephalopathies Advisory Committee in June, 2009. Although the lowest estimated annual per-person risk has risen 5 to 18-fold, it may still be as low as 1 in 12 million and the maximal estimated risk remains unchanged at 1:12,000, the FDA still regards the risk to US patients to be "extremely small".

Implications of findings of vCJD prions in non MM individuals:

There are several variations at codon 129 of the human PrP gene, resulting in 3 main genotypes, MM, MV and VV, where M signifies methionine and V, valine. To date, all symptomatic cases of vCJD who have been evaluated have been MM homozygous at the 129 codon. Approximately 40% of the UK population has this genotype. However, there have been a number of circumstances (some described above) in which the pathologic prion has been found in asymptomatic individuals with the MV or VV genotype. This has raised two questions. The first is whether there will be a "second wave" of vCJD among individuals with a non MM genotype, perhaps resulting from a much extended incubation period. Second is the question of whether non MM individuals can be infectious carriers of the vCJD prion. This latter concern was included in the newer FDA infectivity model for US-derived plasma derivatives. Both questions await resolution.

Status of interventions against transfusion transmission of TSEs:

No significant progress has been reported in the development of any pre-mortem test that could be used for blood donors or donations, although one of the tests under development has undergone some preliminary clinical evaluatation.

Currently, one manufacturer has an available, CE-marked affinity filter intended for use with leukoreduced red-cell concentrates. The procedure has been evaluated in the UK and Ireland, but no decision has been made with respect to

its implementation. A process has also been developed for use in the manufacture of solvent-detergent treated plasma for transfusion.

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Prepared by TTD Committee, October, 2009.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2009. 9. 16	該当なし	

一般的名称	抗HBs人免疫グロブリン	研究報告の公表状況		公表国
販売名(企業名)	抗HBs人免疫グロブリン「日赤」(日本赤十字社) 抗HBs人免疫グロブリン筋注200単位/1mL「日赤」(日本赤十字社) 抗HBs人免疫グロブリン筋注1000単位/5mL「日赤」(日本赤十字社)		FDA, CBER. Available from: http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/ucm095070.htm	米国

研究報告の概要

○FDA:米国承認血漿由来第Ⅷ因子製剤によるvCJD感染リスクの可能性:概要
・近年、米国承認血漿由来第Ⅷ因子製剤(pdFVIII, Antihemophilic Factor)の投与を受けた血友病Aおよびフォン・ヴィレブランド(vW)病患者の変異型クロイツフェルト・ヤコブ病(vCJD)感染リスクに関する疑問が提起されている。
・リスク評価に基づき、FDA、CDC、NIHを含む米国の公衆衛生総局(PHS)は、pdFVIII製剤の投与を受けた血友病AとvW病患者のvCJD感染リスクは、はっきりとはわからないものの、非常に小さい可能性が最も考えられる。第IX因子製剤を含む他の血漿由来製剤からのvCJD感染リスクは同程度か、更に小さいようである。
・新たな情報を得るためには、血友病治療センターの血友病またはvW病の専門家に連絡するのが良い方法である。
(追加情報)
2003年11月～2007年4月に英国で、赤血球輸血によりvCJDに感染したと考えられる患者4名が発生し、血液製剤のvCJD伝播の可能性について懸念が高まった。このためFDAは、vCJDとBSEの発生率が米国と比べて非常に高い国に渡航した人の供血延期を勧告した。米国では、これまでvCJDを発症した人の血漿から作られたpdFVIII製剤はなく、製剤を投与された人がvCJDを発症したこともない。pdFVIII製剤は、他の血漿由来製剤と比べてvCJD感染因子を多く含むと考えられる。また、血漿由来製剤の製造工程における処理でvCJD感染因子は減少すると考えられる。FDA、CDC、NIHの認識している限り、リスクの最も高い英国を含め、血友病、vW病、その他の血液凝固障害患者がvCJDを発症したという報告はない。FDAはvCJD伝播の可能性低減のため、欧州渡航歴のある人の供血延期など様々な対策を実施している。FDAはpdFVIII製剤のvCJD感染リスクを分析したが、有病率について不明な点が多く正確なリスク評価は不可能である。リスクは非常に小さい可能性が最も考えられるが、ゼロではないだろう。

使用上の注意記載状況・その他参考事項等

抗HBs人免疫グロブリン「日赤」
抗HBs人免疫グロブリン筋注200単位/1mL「日赤」
抗HBs人免疫グロブリン筋注1000単位/5mL「日赤」

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

報告企業の意見	今後の対応
米国食品医薬品局が、米国承認血漿由来第Ⅷ因子製剤の投与を受けた血友病Aおよびフォン・ヴィレブランド病患者のvCJD感染リスクの可能性について、はっきりとはわからないものの、非常に小さい可能性が最も考えられるとの見解を示したとの報告である。生涯反復使用する血漿分画製剤の感染リスクが小さいことは、献血者を米国以上に規制してきた国産製剤はより感染リスクが低いと期待される。	日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある人の献血を制限している。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

Vaccines, Blood & Biologics

Potential vCJD Risk From US Licensed Plasma-Derived Factor VIII (pdFVIII, Antihemophilic Factor) Products: Summary Information, Key Points

Summary Information

Key Points:

- In recent years, questions have been raised concerning the risk of variant Creutzfeldt-Jakob disease (vCJD) (a rare but fatal brain infection) to hemophilia A and von Willebrand disease patients who receive US licensed plasma-derived Factor Eight (pdFVIII, Antihemophilic Factor) products.
- Based on a risk assessment, the US Public Health Service (PHS), including FDA, CDC, and NIH, believes that the risk of vCJD to hemophilia A and von Willebrand disease patients who receive US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. vCJD risk from other plasma derived products, including Factor IX, is likely to be as small or smaller.
- Contacting a specialist in hemophilia or von Willebrand disease at a Hemophilia Treatment Center is a good way to learn about new information as it becomes available.

Additional Information:

- Between December 2003 and April 2007, there have been four reports of people, all in the UK, who probably acquired the vCJD agent through red blood cell transfusions. This has increased concern about the potential transmission of vCJD by blood products.
- Principal concerns are whether persons infected with vCJD could donate plasma in the U.S., and whether clotting factor products made from their plasma donations might transmit the disease.
- To address these concerns FDA recommends the deferral of donors who may have lived in or traveled extensively to countries with a higher prevalence of vCJD and bovine spongiform encephalopathy (BSE) than in the U.S.
- In the United States, pdFVIII products have not been made from the plasma of anyone known to have developed vCJD, and no one who received any of these products is known to have developed vCJD.
- FDA conducted a risk assessment for pdFVIII because the plasma fraction from which it is made is likely to contain more of the vCJD infectious agent, if present, than plasma fractions from which other plasma-derived products are made, such as Factor IX, (used to treat hemophilia B), albumin, and immune globulins. The FVIII-containing fraction is further processed using a variety of methods that are likely to reduce or

potentially eliminate vCJD from the final pdFVIII product. Methods likely to reduce or potentially eliminate vCJD are also used in the manufacture of other plasma-derived products.

- FDA, CDC, and NIH are not aware of any cases of vCJD having been reported worldwide in patients with hemophilia, von Willebrand disease, or other blood clotting disorders. This includes those who have received, over a long period of time, large amounts of blood clotting factor products manufactured from plasma donations from the UK where the risk of vCJD is highest because of a previous higher risk of potential exposure to BSE-infected beef in the UK diet.
- The FDA has taken a number of steps to further reduce the potential vCJD risk from blood components. These steps include donor deferral recommendations, and quarantine and withdrawal of products at increased vCJD risk. Donor deferral guidance, first issued in August 1999 and subsequently updated, includes, among other things, deferral of donors who visited or resided in Europe where BSE prevalence is higher than in the US. Also, blood components and plasma derivatives are to be withdrawn if a donor is later diagnosed with vCJD. The potential spread of vCJD through red blood cell or plasma transfusion is limited by these deferral and quarantine measures that are in place.
- Additional steps FDA is taking to reduce potential vCJD risk from plasma derivatives include gathering, evaluating, and disseminating information about manufacturing processes that potentially could reduce the vCJD infectious agent in blood products. FDA is helping to develop donor screening and diagnostic tests for vCJD, and to inform patients and physicians about the current scientific understanding of vCJD risk from blood products.
- Using a computer model, FDA assessed the potential risk of vCJD infection from the current use of pdFVIII products. However, because so much is unknown about vCJD and its prevalence, the risk assessment performed by FDA has a lot of uncertainty, making it impossible to precisely estimate the risk of vCJD in general, or of the actual risk to individual hemophilia A or von Willebrand disease patients. Meaningful distinctions also could not be made among specific products. There is no test yet available to detect vCJD infection in healthy donors or recipients.
- Although the risk of vCJD exposure from US pdFVIII products is most likely to be extremely small, it may not be zero, and FDA is encouraging physicians and patients to consider this risk, in the context of all remaining real or potential risks and the known benefits of product use, when making treatment decisions.
- At this time, the PHS does not believe there is a need for hemophilia A and von Willebrand disease patients who receive pdFVIII to inform their surgeons or dentists about their potential exposure to vCJD. Also, there is no recommendation for surgeons and dentists to take any special precautions based on such potential exposures. This belief is based on the results of the FDA risk assessment, as well as on the lack of known cases of vCJD transmitted by plasma-derived clotting factor products in the UK or anywhere else in the world. PHS agencies will continue to monitor and reevaluate the situation as new information becomes available.
- vCJD originally came from a disease in cattle called "mad cow disease" or

BSE (bovine spongiform encephalopathy) . Transmission of the BSE agent to humans, leading to vCJD, is believed to occur primarily from eating beef and beef products contaminated with the BSE agent. Both BSE and vCJD are invariably fatal brain diseases with incubation periods typically measured in years.

- From 1995 through April 2007, 202 individuals with vCJD were reported worldwide, with 165 in the United Kingdom (UK), and three in the United States. Two of the individuals in the United States had lived in the UK from 1980-1996 during a key exposure period to the BSE agent. The third US individual with vCJD most likely acquired the disease in Saudi Arabia. The reported incidence of vCJD in the UK based on disease onset peaked in 1999 and has been declining thereafter. In the UK, where most cases of vCJD have occurred, the current risk of acquiring vCJD from eating beef and beef products appears to be negligible.
- More information about vCJD is available on these government websites:
  - FDA: Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD) From Plasma-Derived Products
  - Centers for Disease Control and Prevention: vCJD (Variant Creutzfeldt-Jakob Disease)
  - US Department of Agriculture
- Information also may be obtained from these non-government sources:
  - Committee of Ten Thousand
  - Hemophilia Federation of America
  - National Hemophilia Foundation and/or HANDI
  - World Federation of Hemophilia

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- (800) 835-4709
- (301) 827-1800
- ocod@fda.hhs.gov
- **Consumer Affairs Branch (CBER)**

  Division of Communication and Consumer Affairs

  Office of Communication, Outreach and Development

  Food and Drug Administration

  1401 Rockville Pike

  Suite 200N/HFM-47

  Rockville, MD 20852-1448

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

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一般的名称	人ハプトグロビン	研究報告の公表状況	Vox Sanguinis 2009; 97: 207-210	公表国 イギリス
販売名（企業名）	ハプトグロビン静注2000単位「ベネシス」（ベネシス）			

研究報告の概要

（背景）
輸血や血液製剤の投与を介して変異型クロイツフェルト-ヤコブ病(vCJD)が伝播することの公衆衛生上の危険性は、特に血友病患者の体内で異常プリオンタンパク質が検出されたことが最近報告されたことから、現在でも懸念されている。
（目的）
英国のvCJD臨床症例について過去に血漿分画製剤への暴露があったか説明すること。
（方法）
国立CJDサーベイランスユニット(National CJD Surveillance Unit)に保管されている記録(親族、開業医、および病院からのもの)を調査する。
（結果）
英国の168例のvCJD症例のうち9例が、血漿分画製剤の投与をのべ12回受けたことがあった(その12回のうちの1回は1970年でvCJDの危険性以前であったが、残りの11回は1989～1998年であった)。UK CJD Incident Panelの危険性評価基準によれば、11回は低危険度製品の投与であり、1回は低もしくは中等度危険度製品の投与であった。
（結論）
現在までの英国のvCJD臨床症例のうちのいずれの例についても、血漿分画製剤への暴露を介して感染したものとは考えられない。しかし、将来的にそのような伝播がvCJD感染例をもたらす可能性は排除し得ない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 略
1) 略
2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
英国のvCJD臨床症例について過去に血漿分画製剤への曝露があったかについて、国立サーベイランスユニットに保管されている記録を調査した報告である。 血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Variant Creutzfeldt–Jakob disease and exposure to fractionated plasma products

H. J. T. Ward,[1] J. M. MacKenzie,[1] C. A. Llewelyn,[2] R. S. G. Knight,[1] P. E. Hewitt,[3] N. Connor,[4] A. Molesworth[4] & R. G. Will[1]
[1]National CJD Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh, UK, [2]NHS Blood and Transplant, Cambridge Centre, Cambridge, UK, [3]NHS Blood and Transplant, Colindale Centre, London, UK, [4]Health Protection Agency Centre for Infections, London, UK

Background The risk to public health of onward transmission of variant Creutzfeldt–Jakob disease (vCJD) via blood transfusion and plasma product administration is of on-going concern, particularly with the recent reported detection of abnormal prion protein in a person with haemophilia.

Objectives To describe the history of fractionated plasma product exposure in clinical cases of vCJD in the UK.

Methods Through examination of records held at the National CJD Surveillance Unit (from relatives, general practices and hospitals).

Results Nine out of 168 UK vCJD cases had a history of receipt of fractionated plasma products on 12 different occasions (1 pre-vCJD risk in 1970, the remaining between 1989–1998). According to the UK CJD Incident Panel risk assessment criteria, 11 were low-risk products and one was low or medium risk.

Conclusion It is unlikely that any of the UK vCJD clinical cases to date were infected through exposure to fractionated plasma products. However, the possibility that such transmission may result in vCJD cases in the future cannot be excluded.

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Key words: fractionated plasma products, public health, transfusion, vCJD.

Introduction

The risk of onward transmission of variant Creutzfeldt–Jakob disease (vCJD) via blood transfusion and plasma product administration is of on-going concern. This has been highlighted by the recent announcement by the UK's Health Protection Agency of the post-mortem finding of abnormal prion protein in the spleen of a patient with haemophilia who died from a cause unrelated to vCJD [1]. This individual had received UK-sourced fractionated plasma products before 1999, when safety measures were put in place in relation to vCJD, including importation of plasma, mainly from the USA, to manufacture plasma products. There has been no previous documentation suggesting transmission of any type of CJD by fractionated plasma products. On the other hand, variant CJD has been shown to be transmissible via blood component transfusion, with four instances of transfusion-transmitted vCJD infection to date associated with non-leucodepleted red cells [2–5].

Laboratory studies in animal models have shown that infectivity may be present in plasma both during clinical illness and in the incubation period [6]. Although there is experimental evidence that significant infectivity may be cleared during the production process for fractionated plasma products [7], there are doubts about the interpretation of studies that have been largely based on spiking of plasma with brain-derived material rather than endogenous infectivity [8]. In addition, there are varieties of manufacturing processes used in production of plasma products. These findings have drawn attention to the important public health implications of potential secondary transmission of vCJD.

Correspondence: Hester Ward, National CJD Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK.
E-mail: h.ward@ed.ac.uk

In addition to recipients of vCJD-implicated labile blood components, more than 4000 UK-sourced plasma product recipients have been classified and notified by the UK CJD Incidents Panel as 'at risk for public health purposes', in part on the basis of a risk assessment [9]. In 2004, the UK CJD Incidents Panel advised that patients who were treated with UK-sourced fractionated plasma products between 1980 and 2001 and who were exposed to a 1% risk of infection in addition to the background risk of the UK population through diet, should be contacted and advised to take public health precautions. Fractionated plasma products were categorized into three groups according to the number of treatments that were likely to result in a patient reaching this risk threshold: high risk (one treatment with factor VIII, factor IX or antithrombin), medium risk (several infusions of intravenous immunoglobulin or 4·5% albumin) and low risk (intramuscular immunoglobulins or 20% albumin) [1, personal communication]. An exercise was undertaken to trace recipients, estimate individual risk and inform all those who reached this threshold that they were 'at risk of vCJD for public health purposes'. The amount of potential infectivity in the low-risk category was estimated to be so small that the likelihood of surpassing the threshold was extremely unlikely and so individual recipients did not need to be traced or notified.

Data from actual cases of vCJD are important in attempting to determine the potential risk from fractionated plasma products. This paper describes a number of UK vCJD cases reported to have received such products before the onset of illness. The characteristics of the specific plasma products involved suggest that these exposures are most unlikely to have been the source of vCJD in these cases.

Methods

The UK National CJD Surveillance Unit (NCJDSU) routinely collects information on potential risk factors for all cases of vCJD referred to the unit [10], including data on blood transfusion, plasma product administration, vaccination and 'injection' histories. The information is obtained from interviews with relatives of cases and, when available, primary care and hospital records. Where possible, batch numbers of fractionated plasma products were obtained for vCJD cases found to have received such products and compared with the list of product batches derived from plasma donated by individuals who later went on to develop vCJD. Eleven of the 168 cases of vCJD referred to the NCJDSU up to end of March 2009 are known to have made 25 plasma donations which had been used to manufacture 191 batches of fractionated products, prior to the UK importing plasma from abroad in 1999.

Results

To examine whether any of the 168 vCJD cases had received fractionated plasma products, we examined records held at NCJDSU. One hundred and fifty-eight had data available from relatives and general practice/hospital records, seven from relatives only, two from general practice records only and in one case there was minimal information available (this patient was investigated in another country).

Nine cases of vCJD, with onset of symptoms between December 1994 and April 2006, had recorded receipt of fractionated plasma products on 12 occasions (Table 1). Five

Table 1 Variant CJD clinical cases reported to have received plasma products

vCJD case	Plasma product	Year given	Year clinical onset of vCJD	Batch number known (country of plasma origin, if known)
1	Human normal immunoglobulin[a] (gammaglobulin for travel)	1990	1994	✓ (non-UK)
2	Rh(D) immunoglobulin[a]	1992	1995	✗
	Human normal immunoglobulin[a] (gammaglobulin for travel)	1993		✓ (non-UK)
3	Rh(D) immunoglobulin[a]	1991	1996	✓ (UK)
4	Albumin	1993	1998	✗
5	Rh(D) immunoglobulin[a]	1989	1998	✗
	Rh(D) immunoglobulin[a]	1993		✓ (UK)
	Rh(D) immunoglobulin[a]	1998		✗
6	Human normal immunoglobulin[a] (gammaglobulin for travel)	1993	1999	✓ (non-UK)
7	Human normal immunoglobulin[a] (for travel)	1991	2000	✓ (UK)
8	Rh(D) immunoglobulin[a]	1970[b]	2001	✗
9	Rh(D) immunoglobulin[a]	1997	2006	✗

[a]Administered intramuscularly; [b]before the considered start of the vCJD at risk period in 1980.

cases had received Rh(D) immunoglobulin to protect against Rh isoimmunization, four in childbirth (on six occasions) and one (case 9, Table 1) with receipt of fresh frozen plasma. Before travel, four cases had received normal human immunoglobulin for intramuscular use (three gammaglobulin, one human normal immunoglobulin), including one case (case 2, Table 1) who had received Rh(D) immunoglobulin previously. The remaining case was given albumin (unknown concentration) for 'cover' during a paracentesis procedure. One of the nine cases received Rh(D) immunoglobulin in 1970 before the considered start of the vCJD at risk period (1980) and the other eight received products between 1989 and 1998.

Batch numbers were available for only two of the seven Rh(D) immunoglobulin products, which indicated the UK as origin of the plasma in these two cases. However, batch numbers were available for all four intramuscular human normal immunoglobulin/gammaglobulin products and one of these was of UK origin. The albumin batch number was not recorded. No batch number matched any others, nor did the batch numbers match any of those from products known to have included plasma donated from individuals who subsequently went onto develop vCJD.

Discussion

Of the nine vCJD patients who had received fractionated plasma products, the batch numbers of the plasma products, where known, did not correlate with any of the batches derived from pools containing a donation from a person who went on to develop vCJD. Eight had received products considered by the UK CJD Incidents Panel as low risk and one person had received a low-/medium-risk product (albumin of unknown concentration). It is, therefore, unlikely that administration of plasma product was the source of vCJD infection in these cases.

Thirty-two of the 74 female vCJD cases had children and, of these, four (13%) were reported to have received Rh(D) immunoglobulin. In the UK, 17% of all women are RhD negative. Approximately 10% of all UK pregnancies are in RhD negative mothers bearing RhD positive babies, and these women should all receive routine Rh(D) immunoglobulin after delivery [11]. Although less likely now, in the past RhD negative women may have been given Rh(D) immunoglobulin without the blood group of the baby being known, resulting in more than 10% receiving Rh(D) immunoglobulin. However, the median age at death in vCJD is only 28 years and the proportion of women with vCJD who received Rh(D) immunoglobulin is comparable to the likely exposure rate in the general population.

The lack of evidence of transmission of vCJD through fractionated plasma products assumes that accurate and thorough information has been obtained on relevant exposure [10]. Information was available from relatives, hospital notes at the time of admission for the terminal illness and from primary care records in 158 of the 168 cases included in this analysis. In this group, it is unlikely that any plasma product exposure was missed and, in particular, it is unlikely that higher-risk exposures involving long-term treatment with plasma products, such as treatment of haemophilia, were undetected. This is probably also true for the seven cases in which information from relatives was the only source of data on past exposures. However, it is possible that prior treatment, for example with albumin or intravenous immunoglobulin, could have been missed and it was only possible to identify batch numbers in half of plasma products identified as having been used in vCJD cases. There is also the possibility that infection via plasma products might result in a protracted incubation period because of the relatively low dose exposure and that cases of vCJD infected through this mechanism have yet to occur.

In conclusion, it is unlikely that any of the UK vCJD clinical cases to date were infected through exposure to fractionated plasma products. However, the possibility that such transmission may result in vCJD cases in the future cannot be excluded.

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B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料3において集積報告を行っているため、添付していない）。

血対課ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用
100071	2009/12/17	90805	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	有	無
100072	2009/12/17	90806	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	有	無

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第13回	13-1	臨床検査	Ｃ型肝炎陽性	米国	男性	65歳	2009/09	未回復	症例報告	当該製品	識別番号：09000017（完了報告） 報告日：2009年11月5日 MedDRA: Version(12.1)
	13-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	32歳	2009/07/12	未回復	症例報告	当該製品	識別番号：09000013（完了報告） 報告日：2009年9月24日 MedDRA: Version(12.1)
	13-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	40歳	2009/05	回復	症例報告	当該製品	識別番号：09000012（完了報告） 報告日：2009年8月19日 MedDRA: Version(12.1)
	13-4	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	37歳	2009/04/23	未回復	症例報告	当該製品	識別番号：09000014（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)
	13-5	臨床検査	Ｂ型肝炎抗体陽性	米国	不明	新生児	2009/04/23	未回復	症例報告	当該製品	識別番号：09000015（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第12回	12-1	感染症および寄生虫症	肝炎ウイルスキャリアー	米国	不明	不明	1993	不明	症例報告	当該製品	識別番号：08000002（完了報告） 報告日：2008年12月22日 MedDRA: Version(11.1)
	12-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	48	2008/12/09	未回復	症例報告	当該製品	識別番号：08000034（完了報告） 報告日：2008年1月19日 MedDRA: Version(11.1)
	12-3	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	不明	不明	症例報告	当該製品	識別番号：09000004（完了報告） 報告日：2008年5月18日 MedDRA: Version(12.0)
第11回	11-1	臨床検査	Ｂ型肝炎抗体陽性	米国	男性	17	2008/05	不明	症例報告	当該製品	識別番号：08000007（完了報告） 報告日：2008年6月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（追加報告） 報告日：2008年11月12日 弟11回症例番号11-2において10月17日に報告したものの追加報告 MedDRA: Version(11.1)
	11-2	感染症および寄生虫症	Ｃ型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（完了報告） 報告日：2008年10月17日 MedDRA: Version(11.0)
	11-3	感染症および寄生虫症	Ｂ型肝炎	スペイン	女性	不明	2008/6/3	未回復	症例報告	外国製品	識別番号：08000026（完了報告） 報告日：2008年10月31日 MedDRA: Version(11.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回		0*	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第9回		0	0	0	0	0	0	0	0	0	
第8回		0	0	0	0	0	0	0	0	0	
第7回	7-1	臨床検査	ＨＩＶ抗体陽性	米国	不明	小児	不明	不明	症例報告	外国製品	識別番号： 06000022 (完了報告) 報告日：2006年8月24日 MedDRA: Version(9.0)
第6回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456(追加報告) 報告日：2006年2月15日 第6回症例番号5-1は前回報告における第5回症例番号5-1において報告したものの追加報告 MedDRA: Version(8.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第５回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号：05000456(追加報告) 報告日：2005年11月11日 MedDRA: Version(8.1)
	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号：05000456(完了報告) 報告日：2005年10月27日 MedDRA: Version(8.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2002/11/19	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	1-3	感染症および寄生虫症	Ｂ型肝炎	米国	男性	26歳	2002/10/4	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号：05000001(追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第４回	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001 (追加報告) 報告日：2005 年 4 月 25 日 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001 (完了報告) 報告日：2005 年 4 月 7 日 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001 (追加報告) 報告日：2005 年 4 月 25 日) MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001 (完了報告) 報告日：2005 年 4 月 7 日 MedDRA: Version (8.0)
	4-2	感染症および寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	症例報告	外国製品	識別番号： 04000129 報告日：2005 年 3 月 31 日) MedDRA: Version (8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第３回	3-1	感染症および寄生虫症	Ｃ型肝炎	米国	女性	37歳	2004/5/21	不明	症例報告	当該製品	識別番号：04000023 報告日：2004年6月30日 MedDRA: Version(7.0)
	3-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	63歳	2004/7/27	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-2	臨床検査	Ａ型肝炎抗体陽性	米国	女性	63歳	2004/8/16	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-3	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)
	3-3	臨床検査	Ａ型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第２回	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2003/8/30	軽快	症例報告	当該製品	識別番号：03000006 報告日：2004年1月7日 第1回症例番号 1-3 において報告したもの（FAX報告）の完了報告 MedDRA: Version(6.1)
	2-2	感染症および寄生虫症	Ｃ型肝炎	ドイツ	女性	6歳	1994/6/21	未回復	症例報告	外国製品	識別番号：04000013 報告日：2004年5月27日 MedDRA: Version(7.0)
第１回	1-1	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：D03-31 報告日：2003年8月6日 MedDRA: Version(6.1)
	1-2	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：A03-32 報告日：2003年8月6日 MedDRA: Version(6.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男	26歳	2003/8/30	軽快	症例報告	当該製品	FAX報告 報告日：2003年11月19日 （識別番号：03000006 2003年11月28日） MedDRA: Version(6.1)

100071	2009/12/17	90805	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第13回	13-1	臨床検査	Ｃ型肝炎陽性	米国	男性	65歳	2009/09	未回復	症例報告	当該製品	識別番号：09000017（完了報告） 報告日：2009年11月5日 MedDRA: Version(12.1)
	13-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	32歳	2009/07/12	未回復	症例報告	当該製品	識別番号：09000013（完了報告） 報告日：2009年9月24日 MedDRA: Version(12.1)
	13-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	40歳	2009/05	回復	症例報告	当該製品	識別番号：09000012（完了報告） 報告日：2009年8月19日 MedDRA: Version(12.1)
	13-4	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	37歳	2009/04/23	未回復	症例報告	当該製品	識別番号：09000014（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)
	13-5	臨床検査	Ｂ型肝炎抗体陽性	米国	不明	新生児	2009/04/23	未回復	症例報告	当該製品	識別番号：09000015（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第12回	12-1	感染症および寄生虫症	肝炎ウイルスキャリアー	米国	不明	不明	1993	不明	症例報告	当該製品	識別番号：08000002（完了報告） 報告日：2008年12月22日 MedDRA: Version(11.1)
	12-2	感染症および寄生虫症	C型肝炎	米国	女性	48	2008/12/09	未回復	症例報告	当該製品	識別番号：08000034（完了報告） 報告日：2008年1月19日 MedDRA: Version(11.1)
	12-3	感染症および寄生虫症	C型肝炎	米国	女性	不明	不明	不明	症例報告	当該製品	識別番号：09000004（完了報告） 報告日：2008年5月18日 MedDRA: Version(12.0)
第11回	11-1	臨床検査	B型肝炎抗体陽性	米国	男性	17	2008/05	不明	症例報告	当該製品	識別番号：08000007（完了報告） 報告日：2008年6月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（追加報告） 報告日：2008年11月12日 第11回症例番号11-2において10月17日に報告したものの追加報告 MedDRA: Version(11.1)
	11-2	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号：08000018（完了報告） 報告日：2008年10月17日 MedDRA: Version(11.0)
	11-3	感染症および寄生虫症	B型肝炎	スペイン	女性	不明	2008/6/3	未回復	症例報告	外国製品	識別番号：08000026（完了報告） 報告日：2008年10月31日 MedDRA: Version(11.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回		0*	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第9回		0	0	0	0	0	0	0	0	0	
第8回		0	0	0	0	0	0	0	0	0	
第7回	7-1	臨床検査	ＨＩＶ抗体陽性	米国	不明	小児	不明	不明	症例報告	外国製品	識別番号：06000022（完了報告） 報告日：2006年8月24日 MedDRA: Version (9.0)
第6回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号：05000456（追加報告） 報告日：2006年2月15日 第6回症例番号5-1は前回報告における第5回症例番号5-1において報告したものの追加報告 MedDRA: Version (8.1)

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第5回	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456(追加報告) 報告日：2005年11月11日 MedDRA: Version(8.1)
	5-1	感染症および寄生虫症	Ｃ型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456(完了報告) 報告日：2005年10月27日 MedDRA: Version(8.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2002/11/19	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	1-3	感染症および寄生虫症	Ｂ型肝炎	米国	男性	26歳	2002/10/4	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第４回	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（追加報告） 報告日：2005 年 4 月 25 日 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（完了報告） 報告日：2005 年 4 月 7 日 MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（追加報告） 報告日：2005 年 4 月 25 日） MedDRA: Version (8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6 歳	2005 年	不明	症例報告	当該製品	識別番号： 05000001（完了報告） 報告日：2005 年 4 月 7 日 MedDRA: Version (8.0)
	4-2	感染症および寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	症例報告	外国製品	識別番号： 04000129 報告日：2005 年 3 月 31 日） MedDRA: Version (8.0)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第３回	3-1	感染症および寄生虫症	Ｃ型肝炎	米国	女性	37歳	2004/5/21	不明	症例報告	当該製品	識別番号：04000023 報告日：2004年6月30日 MedDRA: Version(7.0)
	3-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	63歳	2004/7/27	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-2	臨床検査	Ａ型肝炎抗体陽性	米国	女性	63歳	2004/8/16	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-3	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)
	3-3	臨床検査	Ａ型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)

別紙様式第４

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第２回	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26歳	2003/8/30	軽快	症例報告	当該製品	識別番号：03000006 報告日：2004年1月7日 第１回症例番号 1-3 において報告したもの（FAX報告）の完了報告 MedDRA: Version(6.1)
	2-2	感染症および寄生虫症	Ｃ型肝炎	ドイツ	女性	6歳	1994/6/21	未回復	症例報告	外国製品	識別番号：04000013 報告日：2004年5月27日 MedDRA: Version(7.0)
第１回	1-1	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：D03-31 報告日：2003年8月6日 MedDRA: Version(6.1)
	1-2	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：A03-32 報告日：2003年8月6日 MedDRA: Version(6.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男	26歳	2003/8/30	軽快	症例報告	当該製品	FAX報告 報告日：2003年11月19日 （識別番号：03000006 2003年11月28日） MedDRA: Version(6.1)

100072	2009/12/17	90806	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン